Habitat fragmentation of semi-natural grasslands: Impact on species richness, trophic interactions and population genetics

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1. Introduction

1.1 Habitat fragmentation of semi-natural grasslands

Within the last century semi-natural grasslands (alike natural grasslands) have faced significant reductions in area as a consequence of agricultural intensification and the abandonment of historic land-use practices, such as extensive grazing (Krebs et al. 1999; Benton et al. 2003). In Europe the formerly versatile cultural landscape became a more intensively utilized agricultural landscape. Since the middle of the 19th century, massive losses of calcareous grasslands (up to 95%) occurred in the study region “Fränkische Schweiz” and the surrounding area mainly due to forestation, fertilization, the abandonment of historic land use and secondary succession (Böhmer 1994). In southern Germany, the most severe loss of calcareous grasslands occurred after 1960, with a reduction of more than 50% by 1990 (Wallis De Vries et al. 2002). Today, semi-natural calcareous grasslands are highly fragmented but still of high conservation value, as they rank as the most species-rich habitat of flora and invertebrate fauna in central Europe (Wallis De Vries et al. 2002; Krauss et al. 2010). But this species-rich habitat is endangered, and many species have already suffered serious declines due to habitat loss and isolation (Ewers & Didham 2006). The diversity of butterflies and plants in particular has decreased observably with decreasing habitat. But other than butterflies, reduction of flowering plants is minor. In some parts more than 30% of the butterfly species have already been lost during the past 150 years (Maes & Van Dyck 2001; Bourn & Thomas 2002). Reduction in plant diversity is lower, e.g. in Germany 1% of the specialized plant species are extinct and 42% are endangered (Wallis De Vries et al. 2002).

Habitat fragmentation in modelled landscapes means the breaking apart of formerly well connected land leading to higher isolation without habitat loss (Fahrig 2003). In real landscapes however, fragmentation is generally accompanied by habitat loss, when continuous habitat is reduced into several smaller spatially isolated remnants (Young et al. 1996). Thus “habitat fragmentation” combines the effects of the two factors “isolation” (habitat connectivity) and “habitat loss”. The relationship between habitat area and species richness is called the species-area relationship, and has been demonstrated for numerous species guilds (Rosenzweig 1995). Today, habitat loss and fragmentation are known to be major

threats for local, regional and global biodiversity (Saunders et al. 1991; Debinski & Holt 2000), leading to higher extinction rates.

Decreasing habitat connectivity (increasing isolation) and habitat loss do not affect all species in the same way. Some guilds are particularly affected by habitat loss and increasing isolation, such as species at high trophic levels, food specialists, species with poor dispersal abilities, rare species and habitat specialists (Steffan-Dewenter & Tscharntke 2000; Steffan-Dewenter 2003; Krauss et al. 2003; Ewers & Didham 2006). For these habitat specialists the surrounding matrix is hostile. Specialized species furthermore react more sensitively to a changing environment than do species without a specialization. Butterflies and vascular plants are excellent model organisms for fragmentation studies, as many species are restricted to specific habitats like calcareous grasslands and persist as metapopulations (Hanski & Thomas 1994; Petit et al. 2001; Wallis De Vries et al. 2002; Helm et al. 2006; Krauss et al. 2010).

The species-area relationship is based on the assumption that with increasing habitat area the species diversity increases. Studies displaying these effects exist for butterflies (e.g. Steffan-Dewenter & Tscharntke 2000) and other species groups (Ricklefs & Lovette 1999; Helmus et al. 2014). However studies on the effects of habitat connectivity are relatively rare and even more so when disentangled from the effects of habitat area (Öckinger & Smith 2006). Furthermore, most studies consider either single species or complete species groups but only few studies classify species upon their ecological background or life-history traits (Öckinger et al. 2010). In this study, butterfly and plant species were classified as specialist species, when their occurrence was restricted to semi-natural grasslands. Those species have often specific requirements of their habitat, which in turn makes them dependent on one habitat type. Species that are not restricted to one habitat type are classified as generalists. In Chapter 5 the consequences of the independent effects of habitat fragmentation (habitat connectivity and habitat area) on butterflies and plant specialists and generalists will be examined by using a new study design with paired large and small study sites in non-overlapping landscapes covering the whole connectivity gradient in the study region.

In semi-natural grasslands complex interactions and dependencies have evolved for centuries. Trophic interactions have been established on the basis of specialized species may collapse when parts are removed or surrounding conditions are changing (e.g. Didham et al. 1996; Steffan-Dewenter & Tscharntke 1999; Vanbergen et al. 2006; Tylianakis 2007; Martinson & Fagan 2014). Species on the basis of trophic cascades (host species) should be less sensitive to changing surrounding conditions. Whereas species at higher trophic levels (herbivores, parasitoids) should be more sensitive, as they additionally depend on their specific food resources (Herbst et al. 2013). Chapter 6 reveals what happens to a trophic system in a semi-natural grassland with a host plant mainly restricted to this habitat type, a specialist butterfly (herbivore) restricted to the host plant and its parasitoids, in the context of habitat fragmentation.

The results of the second study (Chapter 6) lead us to examine the effects of habitat fragmentation on the genetic structure of a butterfly restricted to semi-natural grasslands. Studies of population genetics are not only popular to address interspecific but also intraspecific relationships (Avise 1994). Former molecular analyses have shown that habitat fragmentation may lead to the subdivision of formerly interconnected populations by an increase in genetic differentiation resulting from reduced gene flow (Keyghobadi et al. 2005; Hill et al. 2006; Dixo et al. 2009). A parallel decline of genetic diversity due to genetic drift is often observed, especially in small and isolated populations (Keyghobadi et al. 2005). It is predicted that a species with high restrictions to a certain habitat type exhibits changes in its genetic structure and genetic diversity when habitat fragmentation increases. Apart from the effects of e.g. habitat area, Chapter 7 addresses the effects of habitat connectivity on the genetic diversity and differentiation of a butterfly with a patchy distribution by using data from microsatellite analyses.

1.2 Study organisms and habitat

1.2.1 Butterflies and flowering plants

In this study butterflies (Lepidoptera) and flowering plants (Spermatophyta) were recorded on semi-natural grasslands in northern Bavaria. Butterflies in this study include Hesperoidea and Papilionidea as well as the diurnal burnet moths (Lepidoptera: Zygaenidae). Butterflies and plants are some of the most studied organisms in Europe and exhibit high numbers of species especially on semi-natural grasslands. Thus they are key objectives in conservation studies (Van Swaay 2002, Wallis De Vries et al. 2002). According to their life-history-traits (ecological background) butterflies and plants were classified as habitat specialists (high affinity to semi-natural grasslands) and habitat generalists (low affinity to semi-natural grasslands, see Chapter 5).

Apart from studies on species groups, the Chalkhill Blue *Polyommatus coridon* was then used to examine parasitism rates and genetic diversity/differentiation of a specialist butterfly in the context of habitat fragmentation. *P. coridon* (Poda 1761) is a lycaenid butterfly which feeds as larvae exclusively on the Horseshoe Vetch *Hippocrepis comosa* (L. 1753), which is also mainly restricted to semi-natural grasslands. *P. coridon* is a univoltine species with high population densities (Weidemann 1995) and is distributed throughout southern and central Europe (Kudrna 2002). It is considered a sedentary to moderately dispersing species (Settele et al. 2000; Asher et al. 2001; Cowley et al. 2001) and a good indicator of habitat quality (Brereton et al. 2008). Because of negative effects on its abundance due to habitat destruction it is on the Red List and classified as “threatened” in Bavaria (e.g. Reinhardt et al. 2009).

Fig. 1 *Polyommatus coridon*, male

Fig. 2 *P. coridon* larvae

1.2.2 Semi-natural grasslands

Calcareous grasslands in northwestern Europe are predominantely semi-natural grasslands (Wallis De Vries et al. 2002). The Gentiano-Koelerietum is the most distributed plant association in the study region belonging to the Mesobromion (Böhmer 1994). Semi-natural grasslands originate mostly from extensive livestock farming decades and centuries ago. Since the middle of the 19th century, massive losses of semi-natural grasslands occurred in the study region mainly due to forestation, fertilization, the abandonment of historic land-use and secondary succession (Böhmer 1994; Wallis De Vries et al. 2002). Nowadays this habitat type is highly threatened in Germany (Riecken et al. 2006). The remaining calcareous grasslands are of high conservation value as they are considered biodiversity hot spots. To maintain the rank of the most species rich habitat type in Europe (Van Swaay 2002; Wallis De Vries et al. 2002), they need tending strategies in order to prevent succession and conserve high species numbers.

Fig. 3 Semi-natural grassland „Oberailsfeld“

1.3 Objective and key elements of this thesis

Using data from numerous semi-natural grasslands, this study focuses on the consequences of habitat fragmentation on butterfly and plant species. First of all the impact of habitat connectivity (isolation) and habitat loss on species richness of specialist and generalist butterfly and plant species will be examined (Chapter 5). Second, trophic interactions of the herbivore *P. coridon* with its larval food plant (host) and its parasitoids are analyzed to investigate effects of habitat fragmentation (Chapter 6). Thereupon a genetic analysis using microsatellites addresses the consequences of habitat fragmentation on the genetic diversity and differentiation of populations of the butterfly *P. coridon* (Chapter 7).

Questions to be addressed in detail in this thesis:

Impact of habitat fragmentation on species richness (Chapter 5)

- Do large study sites have higher species richness and abundance of butterflies and plants than small sites?
- Do habitat specialists suffer more from decreasing habitat area than generalists?
- Do habitat specialist butterfly and plant species benefit from increasing habitat connectivity?
- Based on the assumption that small sites are less likely to maintain viable populations and depend more on immigration from the surrounding patches, does reduced connectivity have stronger effects on species from small sites than from large sites?

Impact of habitat fragmentation on trophic interactions (Chapter 6)

- Do population densities of all trophic levels increase with increasing habitat area and habitat connectivity?
- Are higher trophic levels, particularly parasitoids of *P. coridon*, more strongly affected by reduced habitat area and connectivity than lower trophic levels?

Impact of habitat fragmentation on population genetics (Chapter 7)

- What are the potential negative effects of habitat fragmentation on the genetic diversity and the genetic differentiation of the butterfly *P. coridon* being restricted to semi-natural grasslands?
- In detail, how are potential effects related to:
  (i) habitat size,
  (ii) habitat isolation,
  (iii) population size of *P. coridon*?

2. Synopsis

2.1 Impact of habitat fragmentation on species richness

Habitat fragmentation and the resulting habitat loss and isolation of habitats are known to be deleterious for the biodiversity of semi-natural grasslands (e.g. Fahrig 2003; Ries et al. 2004, Ewers & Didham 2006). As shown in previous studies, connectivity plays a key role for single butterfly species occurrences (Thomas et al. 2001; Öckinger 2006), but significant effects on species richness are lacking (e.g. Wilcox et al. 1986, Steffan-Dewenter & Tscharntke 2000; Krauss et al. 2003; Krauss et al. 2004a; Bisteau & Mahy 2005). In a study design where large and small habitats were arranged as pairs in non-overlapping landscapes with a radius of 2 km (Fig. 4), connectivity and habitat area were considered as independent variables, so effects of the explanatory variables are not superposed by effects of other variables. The disentangled variables of habitat fragmentation were then tested with respect to effects on species richness of butterfly and plant specialists and generalists.

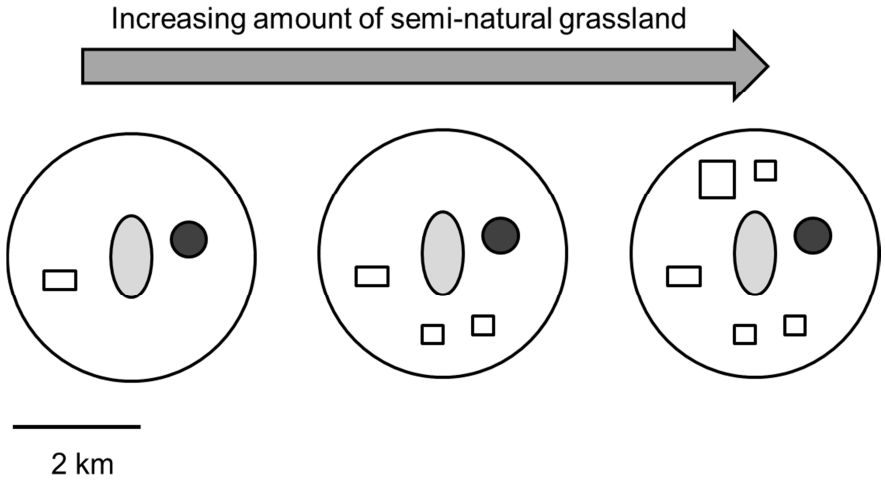


Fig. 4 Schematic study design; the large study site (grey ellipse) of each landscape is located in the middle of the study site and the small study site (small dark grey circle) is nearby, white boxes are further habitat patches. In total 31 non-overlapping landscapes cover the whole connectivity gradient in the study region, ranging from isolated to highly connected study sites.

Butterfly data from 62 study sites (one large and one small per landscape) were sampled from April to end of August in 2007 by visual transect walks (Pollard

1977; Krauss et al. 2003, Westphal et al. 2008). Vascular plants were recorded twice, in May and August 2007. Butterfly and plant species were defined as specialists when restricted to calcareous grasslands (27 butterfly species, 102 plant species), and as generalists when species had no specialization (62 butterfly species, 306 plant species) (for butterflies: Stettmer et al. (2007), confirmed by Weidemann (1995); for plants: local expert in agreement with Gerstberger & Vollrath (2007)).

Increasing connectivity, calculated as Hanski's Connectivity Index (Hanski 1994), had a significantly positive effect on species richness of specialized butterflies and plants (Fig. 5) and also increased the species richness of generalist butterflies (Figure not shown). In contrast to habitat specialists, there was little impact of connectivity on butterfly and plant generalists. As generalists occur in the matrix, an effect of connectivity was not expected.

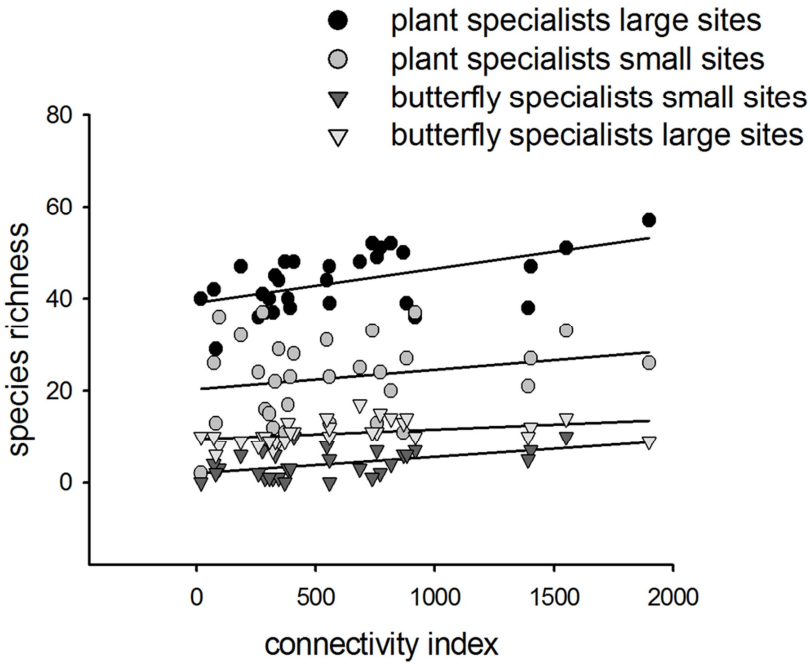


Fig. 5 Butterfly and plant specialist species richness from large and small sites increases with increasing connectivity.

Calculated from the regressions (Fig. 5), on large study sites a high number of specialized butterfly species compared to a moderate number of specialized plant species would be lost if all surrounding habitat patches at a 2 km scale were

destroyed. Compared to large sites, small study sites suffer more from a destruction of surrounding habitat patches (Fig. 6).

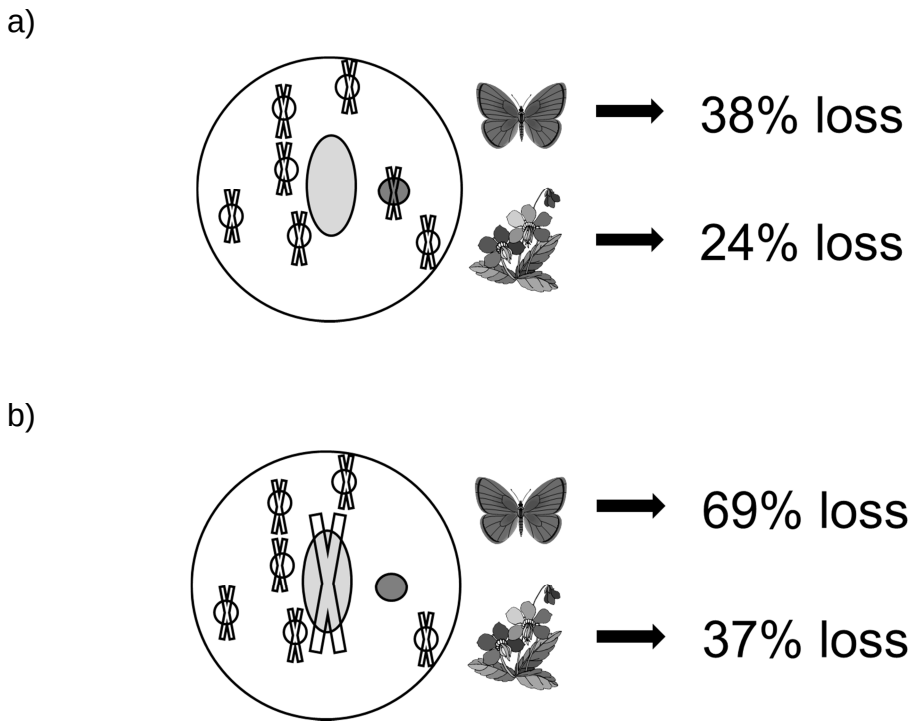


Fig. 6 Percentage loss of specialist butterfly and plant species richness, when on a 2 km scale a) all semi-natural grasslands except for the large study site would be removed, b) all semi-natural grasslands except for the small study site would be removed.

Therefore connectivity of habitats is an important determinant of specialist species richness on large but also on small habitat patches, independent of other habitat factors. These findings are in contrast to previous studies on plant and butterfly species richness (see references above). This might be due to the focus of these studies on species-area relationships, to intercorrelations between area and connectivity or to a partial coverage of regional connectivity gradients. Other studies showed marginally significant effects of connectivity on plant species richness (Adriaens et al. 2006) or an impact of historical landscape connectivity on present day plant diversity (Lindborg & Eriksson 2004; Bommarco et al. 2014), indicating a slow response of plant species richness to fragmentation (Helm et al. 2006; Krauss et al. 2010). Thus, delayed extinction of local plant populations might partly mask the relevance of habitat connectivity for long-living species like plants

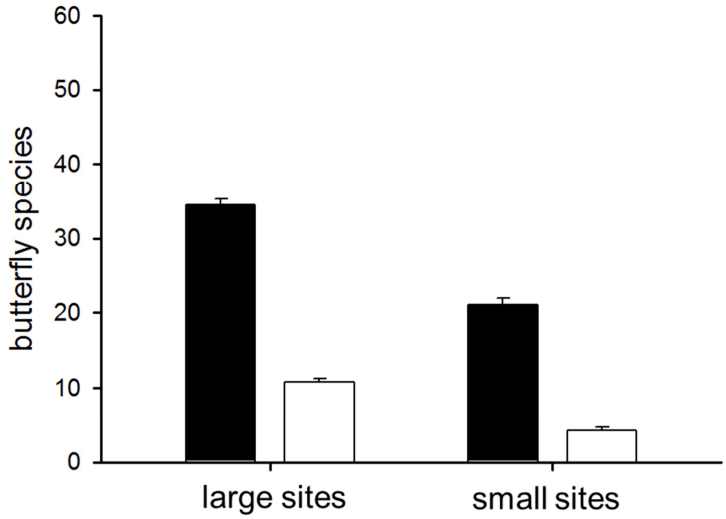
(Kuussaari et al. 2009). Indeed, a more severe species loss for butterflies (up to 69%) than for plants (less than 37%) with decreasing connectivity was found.

Apart from Hanski's Connectivity Index two other possible measures of connectivity explaining species richness of specialist and generalist species were tested: Percent habitat cover, distance to next patch (see Fig. 1 in Chapter 5). Percent habitat cover includes only the amount of area of further semi-natural grasslands on the 2 km radius, but gives no information about the distance from the study sites. Distance to next patch specifies the distance of a habitat patch closest to the study sites, but gives no information about the size of this patch. Statistical analysis yielded Hanski's Connectivity Index to be the best predictor whereas distance to next patch and percent habitat cover did not turn out to be very suitable for data sets from landscapes similar in structure and composition to this study. Distance to the next habitat patch was generally a weak predictor as the small habitat patches scattered around the focal study site might not represent sources for immigration (Boughton 1999). However, for extremely fragmented landscapes or landscapes with a clear mainland-island situation the Euclidian distance to next habitat patch can be useful (Winfree et al. 2005). Habitat cover serves as a good connectivity measure for situations with large proportions of habitats nearby (Winfree et al. 2005; Cozzi et al. 2008). However, habitat cover includes neither the distances from the focal study sites to all the habitat patches in the landscape, nor a scaling of size for the adjacent habitat patches, giving all habitats within a certain radius the same weight (Moilanen & Nieminen 2002). Therefore for community studies that are conducted in similar landscapes we suggest the use of indices that combine distance and patch area of neighboring habitats to quantify differences in habitat connectivity.

The second component of habitat fragmentation, habitat loss, revealed that species richness of both specialist and generalist butterflies and plants were significantly higher in large compared to small study sites (Fig. 7), which is in accordance to other studies' findings (e.g. Krauss et al. 2003) and confirms the general validity of the species-area-relationship (e.g. Wilcox et al. 1986; Rosenzweig 1995; Wettstein & Schmid 1999; Bruun 2000; Zschokke et al. 2000; Krauss et al. 2009; Helmus et al. 2014). NMDS ordination confirmed the second

finding that large sites differ from small sites in their community composition and that habitat area played a major role (Fig. S3, page 80). Clear differences in species richness, species composition and proportion of habitat specialists in small versus large habitat patches suggest that small patches have already lost most butterfly and plant specialists.

a)



b)

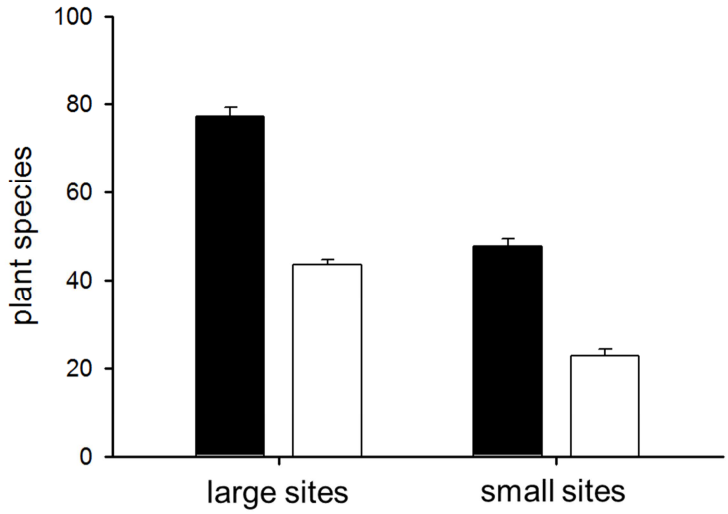


Fig. 7 Number of species differs with habitat size: Species richness of generalist species (black bars) and specialist species (white bars) is higher in large sites compared to small sites for both a) butterflies and b) plants.

The results support the theory of habitat connectivity playing a pivotal role in population ecology. Independent of other habitat factors, isolation, as a result of habitat fragmentation, serves as a driving force in acting against species richness of specialist species of large but especially of small semi-natural grasslands. Additionally, our results confirm the theory of the species-area-relationship (e.g. Wilcox et al. 1986; Rosenzweig 1995). Thus, disruption of habitat connectivity would lead to significant future extinctions of species in addition to extinctions due to habitat loss per se. Conservation management should therefore seek to improve connectivity at a landscape scale. Additionally to conserving large grasslands we recommend adequate landscape management and active restoration of patches of former calcareous grasslands (e.g. by removing trees and bushes) in order to increase habitat connectivity. This will be particularly important for mitigation of possible extinction debt and the long-term survival of habitat specialists in highly fragmented semi-natural grasslands in Europe.

2.2 Impact of habitat fragmentation on trophic interactions

Based on the results from the previous study that habitat isolation and habitat area per se have a significant impact on butterfly specialist species richness, it is of interest whether these effects can be transferred to further trophic levels in a system of the monophagous specialist butterfly *Polyommatus coridon*. Based on the hypotheses that population density of all trophic levels increases with increasing habitat area and connectivity and the assumption that higher trophic levels (particularly parasitoids) are more strongly affected by both factors than lower trophic levels (Fig. 8), data from 17 study sites were analyzed. *P. coridon* larvae were collected in spring (May/June 2008) and were reared in petri-dishes until emergence of an adult butterfly, a parasitoid or until death. The population size of the larval food plant *H. comosa* was estimated when it was flowering in May 2008. Finally the population density of adult *P. coridon* was estimated with transect walks during the peak of the flight season on two days in August 2008.

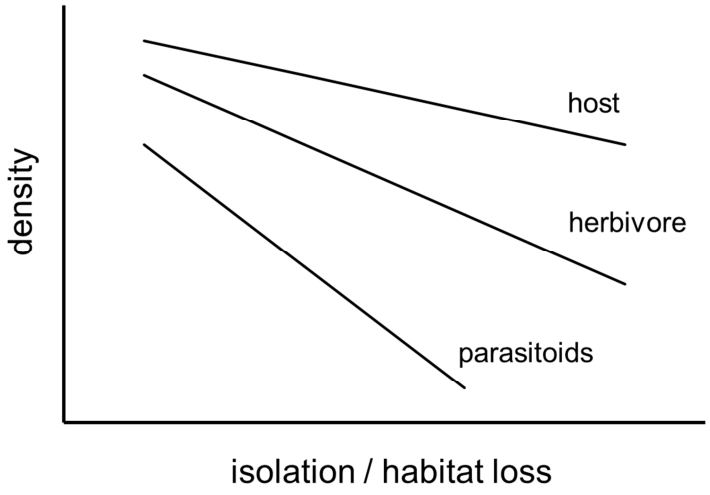


Fig. 8 Hypotheses, that parasitoids and herbivore are more strongly affected by habitat loss and isolation than the plant host.

The Horseshoe Vetch *H. comosa* is the larval food plant of *P. coridon* and serves as the host of the monophagous butterfly. *H. comosa* is at the bottom of the food chain. Population size of *H. comosa* ranged from 35 to 1000 m^2. The population size of *H. comosa* was neither significantly explained by habitat area nor connectivity (see Fig. 1a, b Chapter 6). In contrast to other studies (Krauss et al.

2004a) the amount of the larval food plant did not explain the population density of *P. coridon*. But it is generally assumed that the presence of the larval food plant *H. comosa* is the limiting factor for re-colonization by *P. coridon* (Krauss et al. 2005; Brereton et al. 2008).

Population density of adult *P. coridon* was not significantly affected by habitat area. But population density increased with increasing habitat connectivity (see Fig. 9, taken from Chapter 6), suggesting that *P. coridon* is a sedentary species (Schmitt et al. 2006). For this reason increasing habitat loss and sustained fragmentation may impede between-patch migration leading to genetic impoverishment, inbreeding, loss of fitness and finally to population extinction (Saccheri et al. 1998; Schmitt et al. 2006).

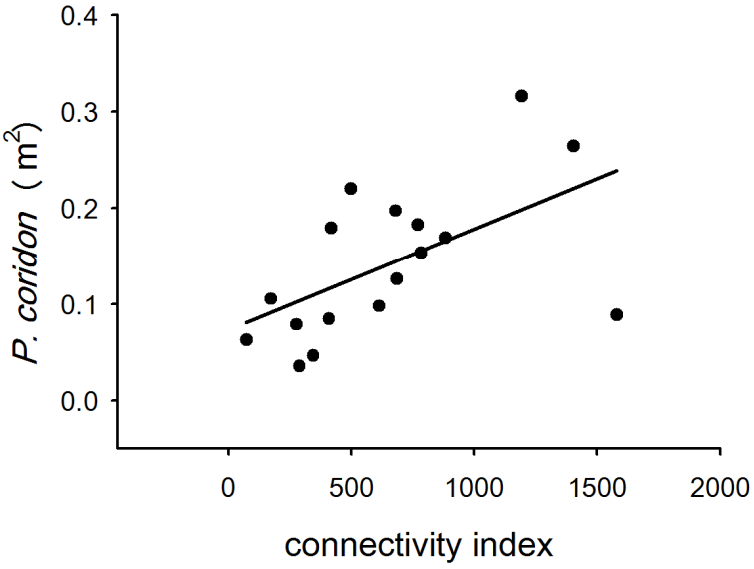


Fig. 9 Population density of *P. coridon* increases with increasing habitat connectivity

In contrast to the study by Krauss et al. (2005) where *P. coridon* was dependent on large habitats with large food plant populations, we found no effect of habitat area within our habitat area gradient (see Fig. 1c in Chapter 6). We therefore assume that the chosen habitat patches had a sufficient patch size to promote viable populations.

The overall rate of parasitism was 10% with five different parasitoid species found belonging to the families of Braconidae, Ichneumonidae and Tachinidae. In contrast to our *a priori* hypothesis, percent parasitism of *P. coridon* larvae did not depend on habitat connectivity or habitat loss (see Fig 1e, f in Chapter 6). A possible explanation is that the parasitoids recorded in *P. coridon* larvae were endoparasites that are not exclusively specialized on *P. coridon*, but feed also on other lycaenid species (Baumgarten & Fiedler 1998; Hinz & Horstmann 2007; Menéndez et al. 2008; Shaw et al. 2009). This leads to the assumption that many parasitoids might be able to use related hosts or even unrelated hosts in similar environments (Shaw et al. 2009). One would expect that parasitism based on parasitoids that are specialized for only one host-species might be strongly affected by increasing isolation of the focal habitat type (Fig. 1), compared to parasitism based on parasitoids with a multi-host-system. Since parasitoids of *P. coridon* can switch between generalist Lycaenidae, extinction of parasitoid species is unlikely even when habitat isolation increases. Alternatively, it might be that more specialized parasitoids of *P. coridon* already became extinct due to habitat fragmentation in the past.

We conclude that other studies (Doak 2000; Cronin 2003; Coudrain et al. 2013), as well as our own, do not generally confirm the prediction that higher trophic levels are more sensitive to habitat loss and isolation than lower trophic levels. One reason is that all recorded parasitoids in our study had a broader host range, while the butterfly host *P. coridon* was highly specialized and restricted to a single larval food plant. We found no impact of habitat area on the abundances of *H. comosa*, *P. coridon* or the parasitism rates. Only habitat connectivity was important for the population density of the studied specialized butterfly species, but not for the host plant or parasitoids. For the conservation of *P. coridon* we suggest a large-scale management of calcareous grasslands that are highly connected with adequate conditions for *H. comosa* to promote persistence of this threatened butterfly species. In contrast, parasitoids, despite their higher trophic level, need less conservation and management efforts, as long as they are able to use other common butterfly species as additional hosts. However, as environmental threats are increasing for all species and as biotic interactions between all species are

unknown, our aim must be to protect valuable habitats like calcareous grasslands and their multi-trophic interactions.

2.3 Impact of habitat fragmentation on population genetics

In the previous studies we described *Polyommatus coridon* as a specialist butterfly species restricted to calcareous grasslands and to be fairly sedentary (Settele et al. 2000; Asher et al. 2001; Cowley et al. 2001) with only few individuals migrating longer distances (Ebert & Rennwald 1991). Furthermore the results from our previous study (Chapter 6) revealed that *P. coridon* suffers from increasing isolation. Despite the patchy distribution *P. coridon* populations reach relatively high abundances, which could be interpreted as a characteristique of a generalist species. As *P. coridon* is ranging somewhere between a strict specialist and a generalist, *P. coridon* also can be considered as an ecologically intermediate species. In this study we tested if the negative effects of habitat fragmentation (habitat connectivity, habitat area) as shown in Chapter 6 can be also found in the population genetics. One would suggest that with increasing habitat isolation and habitat patch size the genetic differentiation of a specialist species increases due to limited gene flow (e.g. Keyghobadi et al. 2005) (Fig. 10).

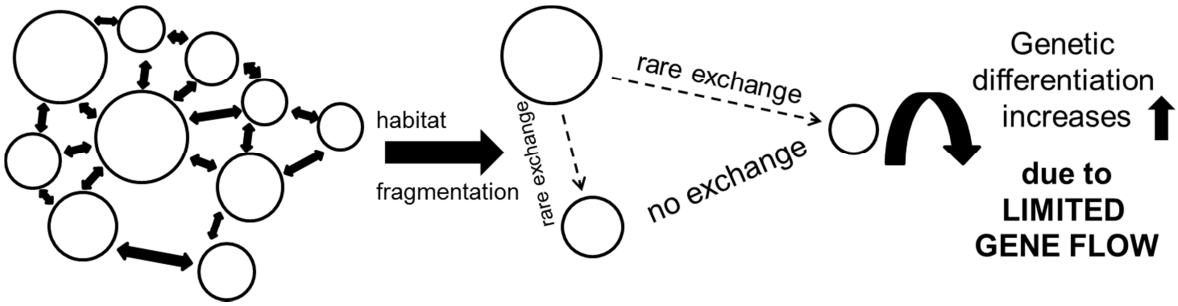


Fig. 10 Highly connected habitats turning into isolated habitats where genetic exchange is rare and thus genetic differentiation is increasing; double arrows = exchange is possible.

In small and isolated populations the genetic diversity can be expected to decrease due to enhanced effects of genetic drift (Keyghobadi et al. 2005; Caplius et al. 2014) (Fig. 11). To test this for *P. coridon* a total of 456 male *P. coridon* individuals from 15 study sites (see Fig. 1 in Chapter 7) in the study region “Fränkische Schweiz” were collected in 2008. The study sites had different habitat sizes, different habitat connectivities and varying population densities. Molecular analysis was conducted using polymorphic microsatellite markers.

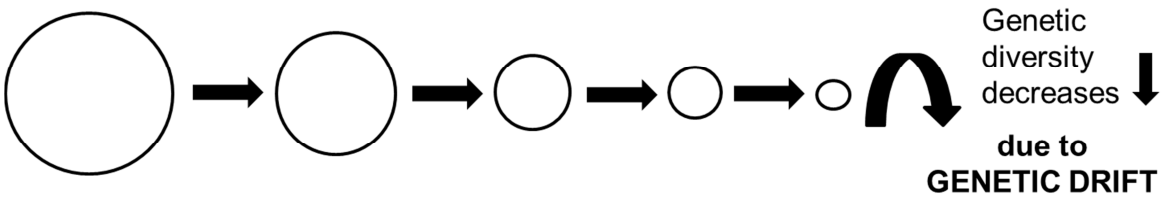


Fig. 11 Habitats becoming smaller and smaller, thus genetic diversity decreases due to genetic drift.

The overall genetic differentiation (AMOVA) was not significant, with a genetic variance of 0.0229 (F_{ST} = 0.0087) found among populations, a genetic variance of 0.3287 (F_{IS} = 0.1261) detected among individuals within populations, and a genetic variance of 2.2774 restricted to single individuals. Pairwise F_{ST} values ranged from 0.0000 to 0.0293 and a lack of isolation-by-distance was found by the Mantel-test. The low genetic differentiation in the present study is a peculiarity of a generalist species. These results are in congruence with a study by Schmitt & Seitz (2002b), who did not find isolation-by-distance on even larger spatial scales, but in contrast to a study by Krauss et al. (2004b), where isolation-by-distance was found at the margin of the distribution range in southern Lower Saxony. Further studies on specialist butterfly species, e.g. *Polyommatus bellargus*, *Parnassius smintheus*, and *Lycaena helle* showed significant isolation-by-distance patterns even on a landscape level (Harper et al. 2003; Keyghobadi et al. 2002; Finger et al. 2009). Here, the lack of isolation-by-distance provides further evidence that (1) habitat isolation does not prevent gene flow between locations, and/or (2) populations are large and thus genetic drift plays a negligible role; further, this result may indicate that (3) habitat isolation in *P. coridon* is very recent and time has not been sufficient to lead to the loss of genetic diversity and genetic differentiation.

Independent factors of habitat fragmentation (habitat connectivity and habitat area) and further habitat parameters showed no significant relationship with any parameter of genetic diversity. Only population density increased significantly with increasing habitat connectivity, which is similar to the results of the previous study (Chapter 6).

Our data for *P. coridon* reveal two inconsistent interpretations: First, the species has relatively strict habitat demands and a patchy habitat distribution, which would suggest a status as a specialist. However, the genetic data show high diversity and low differentiation such as usually found in generalists. Three hypotheses can explain this pattern: (1) The current genetic diversity is the result of relatively large population sizes which prevent genetic drift effects (supported by high Nc estimates of this study, see Table 2 in Chapter 7); (2) few individuals are still migrating among patches preventing differentiation and maintaining high levels of genetic diversity; or (3) the current genetic diversity is a relict of past times when *P. coridon* populations occurred in well-connected grassland habitats in the region. The third scenario would imply that a major proportion of current genetic diversity represents an extinction debt that will be paid in the future. In the latter case, the genetic information still represents the formerly widespread distribution of *P. coridon* when it occurred in most extensively used pastures that in the meanwhile vanished. This condition has been shown for various butterfly species, such as *Zygaena loti*, *Thymelicus acteon* and *Cupido minimus*, that had high levels of genetic diversity, however exist in highly fragmented environments (Habel et al. 2013, with references therein). Yet, even without the use of genetic markers, an extinction debt has been revealed for butterflies for the reason of linkage to long-lived plants (Bommarco et al. 2014).

Our results reveal that *P. coridon* does not match the hypotheses of increasing genetic differentiation and decreasing genetic diversity under the pressure of habitat fragmentation, as *P. coridon* resembles characteristics of both specialist and generalist species. For this “intermediate” species, the actual situation might be a critical signal, as it is not adapted to live in geographic isolation with small population sizes and it can furthermore be suggested that it suffers even stronger under ongoing fragmentation processes than specialist species. That there is a negative correlation of *P. coridon* density with increasing isolation has been shown in this study, and also in the previous study. On a molecular level, *P. coridon* and further species might show an extinction debt of genetic diversity. Thus, conservation actions should take the species-specific population structure and the species’ history into account. It can be speculated whether the current local population sizes and their dispersal capacity are sufficient to counteract genetic

drift and loss of genetic diversity in the future. We recommend an appropriate local and landscape scale management to maintain habitat quality, improve habitat connectivity and preserve the intraspecific variability.

3. Summary (English and German)

3.1 Summary

Habitat fragmentation of semi-natural grasslands, i.e. the combined effects of habitat loss and increasing isolation of habitats of semi-natural grasslands, has been caused by land-use changes throughout central Europe within the last century. Fragmentation in these regions is responsible for the loss of biodiversity and changes in species communities. Species in semi-natural grasslands can be classified in habitat specialists or generalist, depending on life history traits. Effects of habitat fragmentation on specialists and generalists should be different. The present study focuses on three main issues: (1) Consequences of habitat fragmentation on the diversity of butterfly and plant specialists and generalists, (2) impact of habitat fragmentation on trophic interactions of the lycaenid butterfly *Polyommatus coridon* and (3) impact of habitat fragmentation on the genetic diversity and genetic differentiation of *P. coridon*.

The first approach was to look at the impact of habitat connectivity and habitat area (patch size) on species richness of butterfly and plant species, divided into specialists and generalist species. A study design was developed to consider both effects of habitat fragmentation (habitat connectivity, habitat area) independently from each other. The 62 study sites (grassland patches) were arranged in pairs (small and large patches) in 31 non-overlapping independent landscapes covering a large gradient in connectivity for each large and small study site in the study region “Fränkische Schweiz”. Butterfly and plant data were collected in 2007. On the large study sites, 88 butterfly species (31% specialists, 69% generalists) and 379 plant species (26% specialists, 74% generalists) were found. On the small study sites 73 butterfly species (19% specialists, 81% generalists) and 296 plant species (25% specialists, 75% generalists) were recorded. For analysis three different connectivity indices based on different parameters were used: (i) Hanski’s Connectivity Index, combining data of habitat size and distance to the next patch, (ii) percent habitat cover, and (iii) distance to next patch. Species richness of butterfly specialists and generalists as well as plant specialists showed a significant increase with increasing connectivity using Hanski’s Connectivity Index. Furthermore species richness revealed a significantly larger diversity of both butterfly and plant specialists in large sites compared with small sites (see above).

A second approach focused on effects of habitat fragmentation on trophic interactions of a monophagous butterfly in relation with its larval food plant and parasitoids. *P. coridon* feeds as larvae on its host plant the Horseshoe Vetch *Hippocrepis comosa* which is mainly restricted to semi-natural grasslands. The population size of *H. comosa* with a range of 35-1000 m^2 and population density of adult *P. coridon* with a range of 0.04-0.32 individuals per m^2 were recorded in 17 semi-natural grasslands surveyed in 2008. Per site the number of collected *P. coridon* larvae ranged from 20 to 45 individuals, the overall parasitism rate was 10%. None of these variables showed a significant relationship with habitat connectivity or habitat patch size except for population density of adult *P. coridon*. Population density of *P. coridon* significantly increased with increasing connectivity. Parasitoids were probably not affected by changes due to habitat fragmentation as they are not exclusively dependent on the specialist butterfly *P. coridon* but use several (generalist) hosts. Thus, our study does not support the general hypotheses of higher trophic levels being more sensitive to environmental changes. The lower trophic level (*H. comosa*) did not affect population densities of *P. coridon* but as the only larval host plant in this region it restricts the occurrence of *P. coridon*.

Based on the results of the two previous studies, a third approach to consequences of habitat fragmentation on the genetics of the butterfly *P. coridon* was conducted in 15 fragmented landscapes. A total of 456 male individuals (26-32 per site) of *P. coridon* were sampled and eight microsatellites were used for molecular analyses. In the context of habitat fragmentation one would expect a decrease of genetic diversity of the fairly sedentary monophagous butterfly due to genetic drift when habitat patches become smaller. Whereas larger distances between suitable habitats should lead to an increase of genetic differentiation due to limited gene flow between populations. However, *P. coridon* shows characteristics of specialized and generalized species in parallel. Contrary to other habitat specialists *P. coridon* builds up large populations. Further, the genetic analysis exhibited a similar high genetic diversity across the 15 studied populations. The genetic differentiation was low among populations ($F_{ST} < 0.01$). Pairwise F_{ST} revealed a lack of isolation-by-distance. Thus none of the habitat factors had an impact on genetic diversity. Apart from the genetics, only population

density of *P. coridon* negatively correlated with increasing habitat isolation (similar to the results of the second study). As *P. coridon* combines characteristics of both habitat specialists (most ecological characteristics) and generalists (high population abundances, population genetic results) it could be defined as an “intermediate” species. It is speculated that such intermediate species suffer even more from fragmentation than specialist species, as intermediate species are not adapted to persist in geographic isolation. On a molecular level, *P. coridon* might show an extinction debt of genetic diversity that will be paid in the future.

For conservation of species being restricted to semi-natural grasslands we recommend an extensive management on a local and landscape scale, to maintain habitat quality and to improve habitat connectivity. To protect *P. coridon* populations the species-specific population structures as well as the species historical distribution should be taken into account.

3.2 Zusammenfassung

Habitatfragmentierung (zunehmender Habitatverlust und Habitatisolation) von halb-natürlichen Trockenrasen wurde in Mitteleuropa durch Landnutzungsänderungen innerhalb des letzten Jahrhunderts verursacht. Fragmentierung in diesen Regionen ist verantwortlich für den Verlust an Biodiversität und Änderungen in der Artenzusammensetzung. Arten in halb-natürlichen Trockenrasen können gemäß ihrer Life-History-Merkmale (ökologische Merkmale) in Habitatspezialisten und –generalisten eingeteilt werden. Effekte der Habitatfragmentierung für Spezialisten und Generalisten sollen sich dabei unterscheiden. Die vorliegende Studie richtet ihren Fokus auf drei wesentliche Themen: (1) Konsequenzen der Habitatfragmentierung für die Diversität von Tagfalter- und Pflanzenspezialisten und –generalisten, (2) Einfluss der Habitatfragmentierung auf die trophischen Interaktionen des Bläulings *Polyommatus coridon* und (3) Einfluss der Habitatfragmentierung auf die genetische Diversität und die genetische Differenzierung von *P. coridon* Populationen.

In einem ersten Ansatz wurde untersucht, welchen Einfluss die Habitatfragmentierung auf die Artenzahl der in Spezialisten und Generalisten eingeteilten Tagfalter und Pflanzen hat. Um die beiden Effekte von Habitatfragmentierung (Habitatkonnektivität und Habitatgröße) unabhängig voneinander untersuchen zu können, wurde ein Untersuchungsdesign entwickelt, bei dem die 62 Untersuchungsflächen gepaart (je eine große und eine kleine Fläche) in 31 sich nicht überlappenden Landschaften vorliegen. Diese Landschaften decken für große und kleine Untersuchungsflächen jeweils einen sehr großen Konnektivitätsgradienten in der Untersuchungsregion „Fränkische Schweiz“ ab. Die entsprechenden Daten für Tagfalter und Pflanzen wurden in 2007 erhoben. Auf den großen Untersuchungsflächen fanden sich 88 Tagfalterarten (31% Spezialisten, 69% Generalisten) und 379 Pflanzenarten (26% Spezialisten, 74% Generalisten). Auf den kleinen Untersuchungsflächen fanden sich 73 Tagfalterarten (19% Spezialisten, 81% Generalisten) und 296 Pflanzenarten (25% Spezialisten, 75% Generalisten). Für die Analyse wurden drei Konnektivitäts-Indices, basierend auf verschiedenen Parametern, verwendet: (i)

Hanski's Konnektivitäts-Index, welcher Daten von Habitatgröße und Distanz zum nächsten Habitat kombiniert, (ii) prozentualer Anteil an Habitatdeckung in der Landschaft und (iii) Distanz zum nächstgelegenem Habitat. Die Artenzahlen von Tagfalter- und Pflanzenspezialisten zeigten einen signifikanten Anstieg mit steigender Konnektivität bei der Verwendung von Hanski's Konnektivitäts-Index. Des Weiteren zeigte sich eine signifikant höhere Artenzahl von Tagfalter- und Pflanzenspezialisten auf großen im Vergleich zu den kleinen Untersuchungsflächen (vgl. Artenzahlen oben).

Im zweiten Ansatz wurden die Effekte der Habitatfragmentierung auf trophische Interaktionen eines monophagen Tagfalters in Zusammenhang mit seiner larvalen Futterpflanze und seinen Parasitoiden betrachtet. *P. coridon* ernährt sich als Larve von seiner Wirtspflanze *Hippocrepis comosa* (Gewöhnlicher Hufeisenklee), welche überwiegend auf Trockenrasen vorkommt. Die Populationsgröße von *H. comosa* variierte von 35 bis 1000 m^2 und die Populationsdichte von *P. coridon* variierte von 0,04 bis 0,32 Individuen/ m^2 in den 17 halbnatürlichen Trockenrasen, die 2008 untersucht wurden. Die Anzahl der gesammelten Larven von *P. coridon* varriierte zwischen 20 und 45 Individuen, die Gesamt-Parasitierung lag bei 10%. Keine dieser Variablen zeigte einen signifikanten Zusammenhang mit Habitatkonnektivität oder Habitatgröße mit Ausnahme der Populationsdichte von *P. coridon*, welche mit steigender Konnektivität signifikant zunahm. Die Parasitoide waren wahrscheinlich von Änderungen durch die Habitatfragmentierung nicht betroffen, da sie nicht allein von *P. coridon* abhängig sind, sondern auch noch weitere (generalistische) Bläulingsarten als Wirte nutzen. Somit stützt diese Untersuchung nicht die Hypothese, dass höhere trophische Ebenen sensibler auf Umweltveränderungen reagieren. Die unterste trophische Ebene (*H. comosa*) hatte keinen Einfluss auf die Populationsdichte von *P. coridon*, aber als einzige larvale Fraßpflanze in der Region beschränkt sie das Vorkommen von *P. coridon*.

Basierend auf den Ergebnissen der beiden vorherigen Untersuchungen wurden in einem dritten Ansatz die Konsequenzen der Habitatfragmentierung auf die Populationsgenetik des Tagfalters *P. coridon* in 15 fragmentierten Landschaften untersucht. Insgesamt wurden 456 männliche Individuen von *P. coridon* (26-32 pro

Fläche) gesammelt. Acht Mikrosatelliten wurden für die molekularen Analysen verwendet. Im Zusammenhang mit Habitatfragmentierung würde man bei kleiner werdenden Habitaten einen Verlust an genetischer Diversität durch genetische Drift erwarten. Dahingegen führen größere Distanzen zwischen geeigneten Habitatflächen zu einem Anstieg der genetischen Differenzierung, welches auf limitiertem Genfluss zwischen den Populationen dieser als standorttreu eingestuften monophagen Tagfalterart hinweist. Im Gegensatz zu anderen Habitatspezialisten bringt *P. coridon* Populationen mit großen Abundanzen hervor. Des Weiteren zeigten die genetischen Analysen eine ähnlich hohe genetische Diversität über alle 15 untersuchte Populationen. Die genetische Differenzierung war zwischen den Populationen gering ($F_{ST} < 0.01$). Eine Isolation-by-distance durch paarweise F_{ST} ergab sich nicht. Somit hatte keiner der Habitatfaktoren einen Einfluss auf die genetische Diversität. Abgesehen von den genetischen Untersuchungen, zeigte nur die Populationsdichte von *P. coridon* einen negativen Zusammenhang mit steigender Isolierung der Habitate (ähnlich der Ergebnisse der zweiten Untersuchung). Da die Art *P. coridon* Eigenschaften von sowohl Habitatspezialisten (die meisten ökologischen Charakteristika der Art), als auch -generalisten (individuenreiche Populationen, populationsgenetische Ergebnisse) besitzt, wurde sie als intermediär eingestuft. Es wird angenommen, dass die intermediären Arten noch stärker unter Habitatfragmentierung leiden, da sie im Vergleich zu Spezialisten nicht angepasst sind in geographischer Isolation zu leben. Auf dem molekularen Level zeigt *P. coridon* möglicherweise eine „extinction debt“ (Aussterbeschuld), die zu einem Verlust genetischer Diversität in der Zukunft führen kann.

Für die Erhaltung von Arten, die nur auf Trockenrasen vorkommen, empfehlen wir ein extensives Management auf lokaler Ebene und auf Landschaftsebene, um Habitatqualität beizubehalten und Habitatvernetzung zu verbessern. Um Populationen von *P. coridon* zu erhalten, sollten sowohl die artspezifischen Populationsstrukturen, als auch die historische Verbreitung der Art miteinbezogen werden.

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5. Impact of habitat fragmentation on species richness

Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes

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Summary:

1. Calcareous grasslands are diversity hotspots for plant and butterfly species in Europe, but connectivity of these grasslands has been reduced by habitat loss and fragmentation in recent decades. Reduced habitat area leads to biodiversity loss, but the significance of habitat connectivity independent of patch size and habitat quality is unclear.

2. Here we focus on the impact of habitat connectivity on both butterfly and plant species richness and compare (i) a connectivity index, (ii) percentage habitat cover, and (iii) distance to the next suitable habitat patch as alternative measures of connectivity. Species were recorded in 2007 in northern Bavaria (Germany) in paired large and small study sites in 31 independent landscapes.

3. We found that total loss of grassland connectivity would reduce species richness of specialist butterflies (38-69%) and specialist plants (24-37%). A connectivity index combining patch size and distance in the surrounding landscape was a better measure of connectivity than percentage habitat cover or distance to the next suitable habitat.

4. Species richness, species density and abundance of habitat specialist butterflies and plants were significantly higher in large compared to small study sites.

5. *Synthesis and applications.* We conclude that connectivity is highly relevant for conservation of butterfly and plant species with specialized habitat requirements, but the decision as to which connectivity measure is most appropriate depends on patch configuration, landscape context, and study design. We suggest that management strategies should aim to increase connectivity by active restoration of former calcareous grasslands to ensure long-term survival of habitat specialist species.

Introduction

Habitat loss and fragmentation are known to be major threats for local, regional and global biodiversity (Saunders et al. 1991; Debinski & Holt 2000). In Europe, natural and semi-natural grasslands have faced significant reduction in area as a consequence of agricultural intensification and of the abandonment of historic land use practices, such as extensive grazing, within the last century (Krebs et al. 1999; Benton et al. 2003). Today, semi-natural calcareous grasslands are highly

fragmented but still of high conservation value, as they rank as the most species-rich habitat of flora and invertebrate fauna in central Europe (Wallis De Vries et al. 2002). Due to habitat loss and fragmentation, many species have suffered serious declines (Ewers & Didham 2006). The relationship between habitat area and species richness is called the species-area relationship, and has been demonstrated for numerous species guilds (Rosenzweig 1995). However, some guilds are particularly affected by habitat loss and fragmentation, such as species at high trophic levels, food specialists, species with poor dispersal abilities, rare species, and habitat specialists (Steffan-Dewenter & Tscharntke 2000; Krauss et al. 2003; Ewers & Didham 2006).

Butterflies and vascular plants are excellent model organisms for fragmentation studies, as many species are restricted to specific habitats like calcareous grasslands and persist as metapopulations (Hanski & Thomas 1994; Petit et al. 2001; Wallis De Vries et al. 2002; Helm et al. 2006). For these habitat specialists the surrounding matrix is hostile. Increasing habitat fragmentation should therefore reduce species richness of specialists more than that of generalists (Krauss et al. 2003; Krauss et al. 2004), but responses to habitat isolation have been inconsistent (Bruun 2000).

Habitat fragmentation can be defined in a broad sense including habitat loss, connectivity effects and edge effects, or in a stricter sense covering mainly habitat connectivity and edge effects (Fahrig 2003; Ewers & Didham 2006). According to Fahrig (2003), habitat fragmentation per se should be defined independently of habitat loss. However, in real landscapes fragmentation is generally accompanied by habitat loss, in contrast to modelled landscapes, which allow the effects of habitat loss and fragmentation to be distinguished. Therefore it is debatable whether habitat fragmentation *stricto sensu*, which mainly refers to reduced connectivity but also increased edge effects irrespective of habitat area, has a clear negative effect on biodiversity (Fahrig 2003; Ries et al. 2004). Many field studies have not found significant effects of connectivity on species richness in fragmented landscapes (e.g. Wilcox et al. 1986; Steffan-Dewenter & Tscharntke 2000; Krauss et al. 2003; Krauss et al. 2004; Bisteau & Mahy 2005), while connectivity has been shown to play a key role for single butterfly species

occurrence (Thomas et al. 2001; Öckinger 2006) and to be an important factor in addition to habitat quality (Dennis & Eales 1999). One reason for the lack of significant effects of connectivity on species richness might be an unsuitable study design. Connectivity may not have been the main focus of studies or may not have been independent from other factors, such as patch size, edge effects or habitat quality. Furthermore, it remains unclear whether species richness depends more on patch connectivity in small patches than in large patches. Small patches often only maintain populations close to extinction thresholds, which need to be rescued by immigration from nearby source habitats (Hanski et al. 1996; Kuussaari et al. 2009). Other factors like edge effects and landscape matrix might also contribute to changes in species interactions and community dynamics (Fagan et al. 1999; Krauss et al. 2003; Ries et al. 2004; Fletcher et al. 2007).

In this study we used a landscape-scale design, where small and large sites were paired within a connectivity gradient in order to assess connectivity effects independently of patch size and other factors, similar to a Swedish study (Öckinger & Smith 2006). Moreover we considered the whole gradient of connectivity in our study region, whereas in other studies an incomplete cover of regional variation in connectivity might have contributed to non-significant results. Finally there are different possibilities to measure connectivity, such as the Hanski's Connectivity Index (Hanski 1994), the percentage habitat cover or the distance to the next suitable patch (Moilanen & Nieminen 2002; Fahrig 2003; Winfree et al. 2005; Kindlmann & Burel 2008). In our study we tested these three measurement methods in parallel, to answer the questions: "*What are the relative merits of different indices of habitat connectivity? Which of them best predict conservation value?"* currently considered by British ecologists as one of the 100 most important and policy relevant research questions in ecology (Sutherland et al. 2006).

The hypotheses addressed in this study are:

1. Large study sites have a higher species richness and abundance of butterflies and plants than small sites.

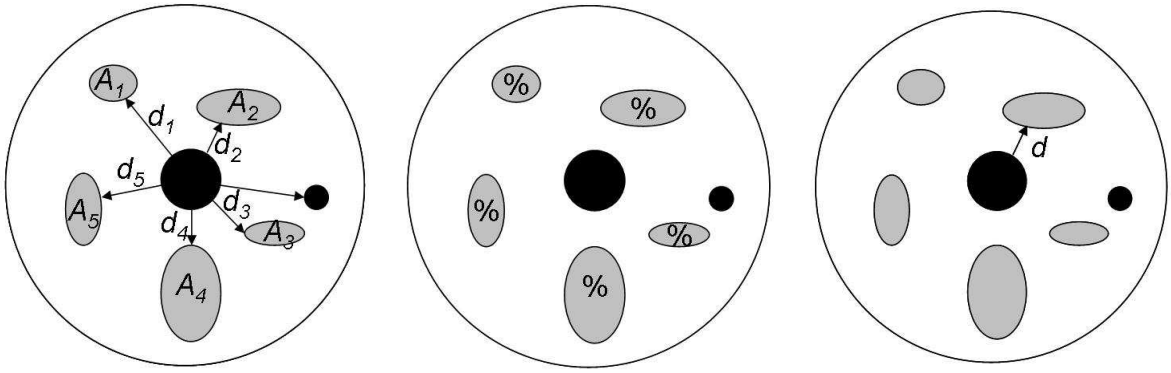
2. Habitat specialists suffer more from decreasing habitat area than habitat generalists.
3. Habitat specialist butterfly and plant species benefit from increasing habitat connectivity.
4. Reduced connectivity has stronger effects on species from small than from large sites, as small sites are less likely to maintain viable populations and depend more on immigration from surrounding patches.

Material and methods

Study region and study sites

The study region “Fränkische Schweiz” is located in the vicinity of the town Bayreuth in northern Bavaria (southern Germany). It is characterized by a bedrock of White Jura including numerous cliffs and semi-natural grasslands (Böhmer 1994). Since the middle of the 19th century massive losses of calcareous grasslands (up to 95%) occurred in the study region mainly due to forestation, fertilization, the abandonment of historic land use, and passive succession (Böhmer 1994). In southern Germany the most severe loss of calcareous grasslands occurred after 1960, with a reduction of more than 50% by 1990 (Wallis De Vries et al. 2002).

A total of 62 calcareous grasslands were selected as study sites, arranged as pairs (large and small patches) within 31 non-overlapping landscapes. The study sites were chosen to cover the whole connectivity gradient in the study region, ranging from isolated study sites to highly connected study sites within a 2 km radius. One large study site in the landscape center (area: 2.4 ± 0.2 ha; perimeter: 1158 ± 88 m) and one small study site close by (area: 0.12 ± 0.02 ha; perimeter: 232 ± 17 m, with an average distance to the large sites of 488 ± 76 m; range 13-1400 m) were located in each landscape (Fig. 1). Landscapes were dominated by agricultural land or pastures (open habitat: 58.4 ± 2.7%) and forest (38.2 ± 2.9%) (CORINE Land Cover data 2000; http://www.eea.europa.eu/themes/landuse/clc-download). Landscapes varied slightly in the amount of open habitat and forest, but increasing area of open habitat (or decreasing forest cover) did not increase species richness of plants or butterflies (results not shown).



a) Hanski's Connectivity Index b) Habitat cover (%) c) Distance to next patch

Fig. 1 Illustration of the three connectivity measures used in this study; black are the focal study sites: one large and one small study sites per landscape, where the large site is in the center of the landscape; a) Hanski's Connectivity Index (see equation in the text), *d* = distance from focal study site to another habitat patch (km), *A* = size of the habitat patch (m^2); b) the total amount of habitat in the landscape (grey) is calculated as percentage habitat cover (habitat cover for the small sites always includes the area of the large study site and habitat cover for the large sites includes the area of the small study sites; the focal study site is always excluded); c) distance to next patch is measured.

For the landscapes where distances between large and small study sites were less than 50 m (n = 4) the patches were additionally separated by a barrier (e.g. forest). Patch size, patch perimeter and distances between sites were calculated with the software ArcView GIS 3.2 (ESRI 1995) using orthorectified digital aerial photos from 2005 for interpretation. Within the study site categories (small or large sites) the gradient of patch size and perimeter were small (Table S1, page 68). Patch size and patch perimeter were highly correlated within small sites (r = 0.60, *P* <0.001) and within large sites (r = 0.87, *P* < 0.001). Connectivity did not correlate with patch size within the categories small or large sites (Table S2, page 68), so that connectivity could be tested independently from size and perimeter in the two habitat area categories (small vs. large sites). As small sites have small perimeters and large sites have large perimeters we cannot distinguish between area and edge effects in this study.

Connectivity and habitat predictors

We calculated and tested three different and often used connectivity measures: 1) Hanski's Connectivity Index (= *CI*) (Hanski 1994), 2) the amount/percentage of calcareous grassland in the landscapes on a 2 km radius (= % *habitat cover*), and 3) the distance from the study site to the nearest calcareous grassland patch in the surrounding landscape (= *distance)*, independently for all 62 small and large study sites. Thereby *CI* and *% habitat cover* are less precise for small compared to large patches, as only the large patches were exactly in the center of the 2 km radius for which detailed land cover data were available.

Hanski's Connectivity Index (= CI) of each study site was calculated by measuring edge-to-edge distances between study site (separately for large and small study sites) and all other habitat patches within the 2 km radius of each landscape (Fig. 1a) using the equation

$$CI_i = \sum_{i \neq j} \exp(-\alpha\, d_{ij})\, A_j^b$$

where A_j is the size (in m^2) of neighboring calcareous grasslands and d_{ij} the distance (in km) from neighboring calcareous grasslands *j* to the study site *i* (Hanski 1994). The parameter α is a measure of the dispersal ability (1/average migration distance in km) and *b* is a parameter, which scales the size of the surrounding habitat patches. We chose α = 0.5, as we expect an average migration distance of 2 km for our butterfly and plant communities (e.g. Moilanen & Nieminen 2002; Adriaens, Honnay & Hermy 2006). We further tested values for α = 0.3 and α = 1 (similarly to Moilanen & Nieminen 2002; Krauss et al. 2003; Helm et al. 2006; Adriaens et al.; Pöyry et al. 2009), but they resulted in highly correlated connectivity measures (pairwise Pearson-correlation, all r > 0.995, all $P < 0.001$). For the scaling parameter *b* we chose *b* = 0.5 according to the assumptions of Moilanen & Nieminen (2002), who suggested that the ratio of patch edge to patch size decreases with $A^{0.5}$ when patch size increases. The connectivity gradient of our landscapes ranged from 18.9 to 1899.6 for large sites and from 103.2 to 1731.7 for small sites.

Percentage habitat cover (Fig. 1b) was measured as the amount of calcareous grassland within each landscape, and calculated separately for each large and small study site. The patch area of each respective focal study site (either large or small site) was excluded. Percentage habitat cover is a landscape connectivity measure especially suitable for landscapes with a high cover of the focal habitat (Winfree et al. 2005; Cozzi et al. 2008). Percentage habitat cover in our landscapes ranged from 0.01% to 2.02% calcareous grasslands for large sites and 0.20% to 2.16% for small sites.

A third measure of connectivity is the Euclidean distance of each study site to the next habitat patch (calcareous grassland) in the surrounding landscape (Fig. 1c), a method regularly used for recolonization events (Boughton 1999), for migration rates (Roland et al. 2000) or the importance of isolation (Bauerfeind et al. 2009). The distance to the next habitat patch ranged from 4 m to 1400 m, and 35% (n = 22) of the 62 study sites were less than 50 m from the next suitable habitat patches with a minimum area of 50 m². We therefore reanalysed the data using only study sites with distances greater than 50 m without a barrier or 20 m with a barrier (e.g. forest), but results did not change consistently.

The Hanski's Connectivity Index was positively correlated with percentage habitat cover for large and small study sites. Also the distance to the next patch was significantly negatively correlated with the Hanski's Connectivity Index and percentage habitat cover (Table S2). We used habitat specialists as well as generalists in the statistical analyses with connectivity measures. Even though generalists were not expected to be restricted to the focal habitat type, or closely linked with measured connectivity values, the comparison of the two species groups provides a useful reference point.

All study sites were selected to be similar in habitat quality within their habitat category (small / large). Nevertheless factors influencing habitat quality such as management practice, percentage bush cover, and percentage flower cover (estimated by pooling the cover of flowering plants per site for the eight transects) were recorded (Table S1 see 68). Of the large study sites 21 were managed, either by sheep grazing (20 sites) or by mowing (1 site), whereas 10 sites were not

managed in the study year 2007. Management of the small study sites was not always clear, as some sites close to roads were incidentally mown and others were grazed by sheep or were fallows. Therefore management could not be considered for small sites. None of the habitat quality factors was correlated with connectivity measures or showed a significant effect on either butterfly or plant species richness (Table S2, S3, page 68f).

Butterflies

Butterflies (Lepidoptera: Hesperioidea and Papilionoidea) and burnet moths (Lepidoptera: Zygaenidae) were sampled from April to the end of August in 2007 by variable visual transect walks (Krauss et al. 2003; Westphal et al. 2008). Species were recorded within a 5 m corridor when weather conditions were suitable for butterfly activity (Pollard 1977). The length of transect walks was approximately 800 m and the duration was 40 min on large study sites, and approximately 200 m and 10 min on small study sites. Transect distance and transect time were measured with a GPS (eTrex Vista, Garmin). All 62 study sites were sampled eight times during summer 2007 in every 2nd to 3rd week. Butterfly counts were separated in 50 m sub-transects to calculate accumulation curves and species saturation. Butterfly individuals were netted and either released immediately or collected for genitalisation when necessary for identification. Genitalisation was necessary to distinguish between *Zygaena minos* and *Z. purpuralis,* between *Z. filipendulae* and *Z. angelicae*, between *Melitaea britomartis*, *M. aurelia*, and *M. athalia*, and between *Polyommatus icarus* and *Polyommatus thersites* as well as between rare individuals of the family *Hesperiidae*. We did not distinguish between *Leptidea reali* and *L. sinapis* or between *Colias alfacariensis* and *C. hyale*. Identification and nomenclature followed Naumann et al. (1999) for burnet moths and Settele et al. (2005) for butterflies. In the following, butterflies always include burnet moths.

Butterflies were defined as strict habitat specialists (n = 27) when the species are only found in calcareous grasslands in Bavaria, according to Stettmer et al. (2007) and confirmed by Weidemann (1995). Butterfly species not specialized for calcareous grasslands were defined as generalists (n = 62), even though this category includes other grassland or forest specialists (Table S4, page 70f).

Butterflies were summed up from the eight conducted butterfly surveys per site on the basis of the transect walks and abundance was calculated as density per 100 m^2.

Plants

Vascular plants were recorded twice, in May and August 2007. Surveys were conducted by two different botanists, one covering the spring period, one the summer period. On the large sites plant species were recorded in 16 random $2m^2$ plots, whereas plant species on the small sites were recorded in 4 random $2m^2$ plots to cope with the two different size classes of the study sites. Species identification and nomenclature followed Rothmaler (1999). Plants were divided into strict habitat specialists and generalists by a local expert, in agreement with Gerstberger & Vollrath (2007). As with butterflies, plant specialists were defined as strict specialist species when restricted to calcareous grasslands (n = 102) and as generalists when they have no or other habitat preferences (n = 306) (Table S4, page 70f). Plants were summed from the two plant surveys for each site and plant abundance was calculated as the density of plants per 1 m^2.

Statistical analysis

The statistical analyses were performed using the software R 2.9.1 (R Development Core Team 2004). The explanatory variables were: connectivity, habitat area and factors of habitat quality (bush cover, flower cover and management) plus patch size within the two habitat area categories. Transformation of the explanatory variables was not necessary. The response variables were: species richness (area adjusted sample size), species richness (estimated), species density (equal sample size) and species abundance of habitat specialized butterflies and plants as well as butterfly and plant generalists. The response variables met the assumptions of normality and homoscedasticity in the statistical models and were therefore not transformed. In linear mixed effects models landscape identity was included as a random factor, as large and small study sites were nested within the 31 landscapes. Explanatory variables entered the mixed effects models in the following sequence: 1) connectivity predictor (Hanski's Connectivity Index, percentage habitat cover or distance to next patch), 2) habitat area (small vs. large sites), 3) interaction of habitat area (small vs. large

sites) with the connectivity predictor (Hanski's Connectivity Index, percentage habitat cover or distance to next patch). As interactions were not significant, the interaction terms were removed from the final models. To compare the importance of the three connectivity measures we used Akaike Information Criterion for small sample sizes AICc (library bbmle in R) and ranked models with small AICc as better than models with large AICc values (Burnham & Anderson 2002).

Species accumulation curves and species richness estimators were calculated using the software EstimateS version 8 (Colwell 2006). Accumulation curves were asymptotic for large and small patches indicating sufficient sample size for all study sites (Fig. S1, page 77). With our study design using subunits for species records we calculated an estimated species richness per study site separately for butterflies and plants. We used the estimator ACE and divided the recorded species richness by estimated species richness to obtain the species saturation per site. A paired t-test revealed that butterflies, but not plants, showed a significantly higher saturation on small sites compared to large sites. Therefore we also present estimated species richness for butterflies. For species density relationships the sample size of large sites was reduced to the same sampling effort as small sites (first 4 transect units or first 4 plot units).

Multivariate ordination methods with nonmetric multidimensional scaling (NMDS) were used to reveal community patterns in the study sites using the R package Vegan (Oksanen 2009). As dissimilarity indices between sites we used "Bray-Curtis" dissimilarities. Arithmetic means ± one standard error are given in the text.

Results

In total, 89 butterfly and 408 plant species were identified on the 62 calcareous grasslands. On the large patches, a total of 88 butterfly and 379 plant species were found with 22870 individual butterflies and 3741 plant records; on the small patches, we recorded 73 butterfly and 296 plant species with 3805 individual butterflies and 2185 plant records.

On average 45.3 ± 1.1 butterfly species (range: 31 - 57) and 120.7 ± 2.8 plant species (range: 96 - 150) were found on the large patches and 25.2 ± 1.4 butterfly

species (range: 12 - 42) and 70.5 ± 1.9 plant species (range: 48 - 89) were found on the small patches. Large study sites contained 31% specialized butterfly species and 69% generalists respectively, whereas small patches supported just 19% specialists and 81% generalists. The differences were less obvious for plant species with 26% specialist species in large patches and 25% specialists in small patches.

Connectivity

None of the interactions between the connectivity measures and habitat area were significant in the mixed effects models (Table 1) indicating that butterflies and plants from large patches reacted similarly to those from small patches (equal slopes). We therefore excluded the interaction terms from all models (Table 1). As species richness patterns were not significantly related to any of the habitat quality measurements (Table S2, page 68), connectivity effects could be tested independently of habitat quality.

Table 1 (next page) Mixed effect models for butterfly and plant species separately for habitat specialists and generalist species

+ = Relationship: large patches > small patches or positive slope

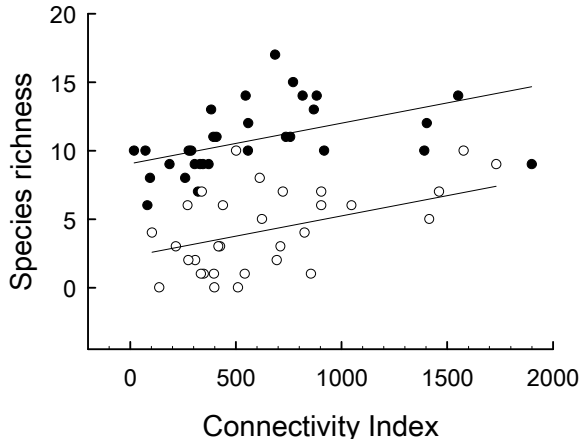
- = Relationship: small patches > large patches (occurs only for generalist plant species)

CI = Connectivity Index; distance = distance to next suitable patch; habitat cover = % of calcareous grassland cover; habitat area = small or large study site

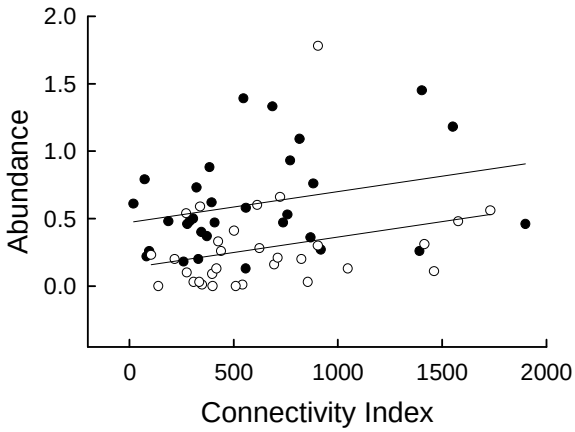
HABITAT SPECIALISTS		Butterflies				Plants			
		Mixed effects model				Mixed effects model			
		$F_{1,29}$	P	AICc		$F_{1,29}$	P	AICc	
Species richness (area adjusted sample size)	CI	11.82	**0.002**		+	5.3	**0.029**		+
	habitat area	119.06	**<0.001**	295.77	+	135.53	**<0.001**	429.03	+
	CI* habitat area		ns				ns		
	habitat cover	0.2	0.659			1.02	0.32		
	habitat area	121.25	**<0.001**	303.37	+	133.34	**<0.001**	429.71	+
	habitat cover* habitat area		ns				ns		
	distance	3.09	0.09			3.12	0.088		
	habitat area	115.91	**<0.001**	306.68	+	130.2	**<0.001**	434.47	+
	distance*habitat area		ns				ns		
Species richness (estimated, ACE)	CI	10.14	**0.004**		+				
	habitat area	24.84	**<0.001**	386.45	+				
	CI* habitat area		ns						
	habitat cover	1.92	0.177						
	habitat area	26.18	**<0.001**	392.03	+				
	habitat cover* habitat area		ns						
	distance	3.87	0.059						
	habitat area	22.75	**<0.001**	393.36	+				
	distance*habitat area		ns						
Species density (equal sample size)	CI	15.09	**<0.001**		+	4.81	**0.037**		+
	habitat area	37.84	**<0.001**	286.56	+	5.48	**0.026**	435.91	+
	CI* habitat area		ns				ns		
	habitat cover	2.4	0.132			6.37	**0.017**		+
	habitat area	38.22	**<0.001**	295.14	+	7.14	**0.012**	433.13	+
	habitat cover* habitat area	0	ns				ns		
	distance	1.98	0.17			1.09	0.306		
	habitat area	33.46	**<0.001**	299.88	+	4.83	**0.036**	440.08	+
	distance*habitat area		ns				ns		
Abundance	CI	4.29	**0.047**		+	2.8	0.105		
	habitat area	14.83	**<0.001**	51.4	+	8.59	**0.007**	442.44	+
	CI* habitat area		ns				ns		

HABITAT GENERALISTS		Butterflies				Plants			
		Mixed effects model				Mixed effects model			
		$F_{1,29}$	*P*	AICc		$F_{1,29}$	*P*	AICc	
Species richness (area adjusted sample size)	CI	4.99	**0.033**		+	0.04	0.847		
	habitat area	127.51	**<0.001**	379.27	+	116.22	**<0.001**	478.85	+
	CI* habitat area		ns				ns		
	habitat cover	1.73	0.198			3.43	0.074		
	habitat area	120.49	**<0.001**	386.61	+	112.28	**<0.001**	479.04	+
	habitat cover* habitat area		ns				ns		
	distance	1.27	0.268			0.02	0.902		
	habitat area	123.4	**<0.001**	386.02	+	115.77	**<0.001**	479.01	+
	distance*habitat area		ns				ns		
Species richness (estimated, ACE)	CI	7.42	**0.011**		+				
	habitat area	58.77	**<0.001**	424.4	+				
	CI* habitat area		ns						
	habitat cover	0.01	0.905						
	habitat area	54.55	**<0.001**	432.4	+				
	habitat cover* habitat area		ns						
	distance	1	0.326						
	habitat area	52.78	**<0.001**	432.92	+				
	distance*habitat area		ns						
Species density (equal sample size)	CI	4.04	0.054		+	2.38	0.134		
	habitat area	7.42	**0.011**	372.53	+	4.38	**0.045**	478.27	-
	CI* habitat area		ns				ns		
	habitat cover	0.03	0.867			2.49	0.126		
	habitat area	6.73	**0.015**	377.09	+	5.34	**0.028**	477.27	-
	habitat cover* habitat area		ns				ns		
	distance	1.09	0.306			0.92	0.347		
	habitat area	6.77	**0.015**	376.09	+	3.97	0.056	480.14	
	distance*habitat area		ns				ns		
Abundance	CI	1.71	0.201			1.87	0.182		
	habitat area	15.47	**0.001**	81.29	+	6.13	**0.019**	444.24	-
	CI* habitat area		ns				ns		

a) Butterfly specialists



b) Butterfly specialists



c) Plant specialists

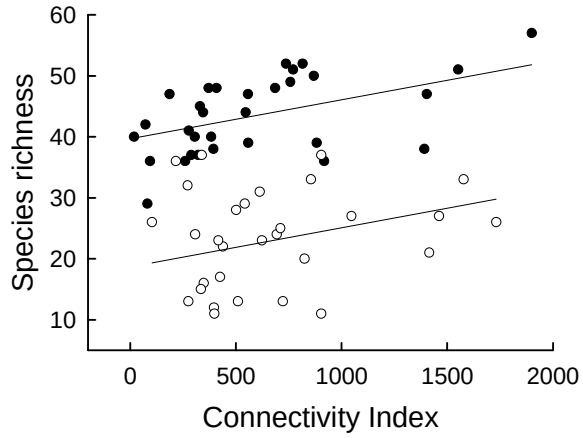


Fig. 2 Butterfly specialist species richness; butterfly specialist abundance and plant specialist species richness in relation to Hanski's Connectivity Index. a) butterfly specialist species richness from large (y = 0.002962x + 9.04) and small sites (y = 0.002962x + 2.28), b) butterfly specialist abundance from large (y = 0.000229x + 0.47) and small sites (y = 0.000229x + 0.13), c) plant specialist species richness from large (y = 0.006410x + 39.66) and small sites (y = 0.006410x + 18.66).

Increasing connectivity, calculated as Hanski's Connectivity Index, had a significantly positive effect on species richness of specialized butterflies and plants (Fig. 2a, c) and also increased the species richness of generalist butterflies (Table 1). Based on this relationship, on large study sites 38% of specialized butterfly species and 24% of specialized plant species would be lost if all surrounding habitat patches at a 2 km scale were destroyed. For small study sites up to 69% of

specialist butterfly and 37% of specialist plant species could be lost (calculations based on regressions from Fig. 2a, c). Connectivity effects were similar for species richness, estimated species richness and species density (Table 1). The abundance of specialized butterfly species increased with increasing connectivity, but neither the abundance of generalist butterfly species nor that of plants was affected by connectivity (Fig. 2b, Table 1).

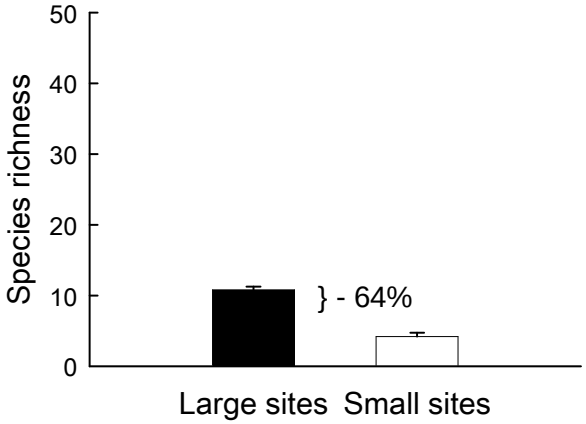
Comparing the three alternative connectivity measures, only Hanski's Connectivity Index was significant for species richness patterns for specialists: models had a lower AICc-value than models with habitat cover or distance to next patch (Table 1). For plant species density, habitat cover was as good a predictor of Hanski's Connectivity Index (Table 1).

Habitat area

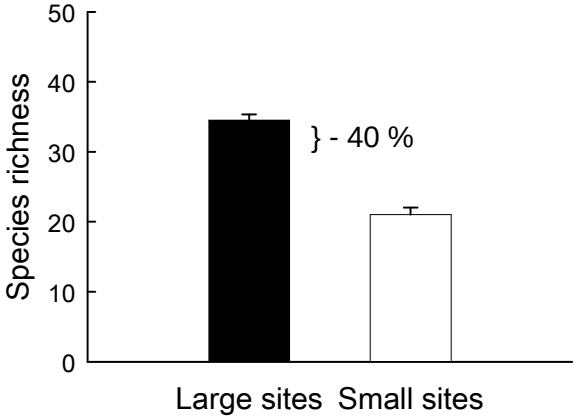
Species richness, estimated species richness, species densities and abundance of both specialist and generalist butterflies were significantly higher in large compared to small study sites (Table 1, Fig. 3; Fig. S2, page 78). Species richness and species density of plant specialists as well as species richness of plant generalists were also significantly higher in large sites, but species density of plant generalists and abundance of plant generalists were higher on small sites (Table 1, Fig. 3; Fig. S2, page 78).

We found 64% fewer butterfly specialists and 40% fewer butterfly generalists as well as 47% fewer plant specialists and 38% fewer plant generalists on small compared to large sites (Fig. 3a, b). Patterns were similar for estimated species richness for butterflies (Fig. 3c, d). Also species density patterns were similar, showing 45% lower butterfly specialist density and a 12% lower butterfly generalist density as well as a 16% lower plant specialist density (Fig. 3e, f, i). However plant generalist density was 14% higher on small compared to large sites (Fig. 3 j).

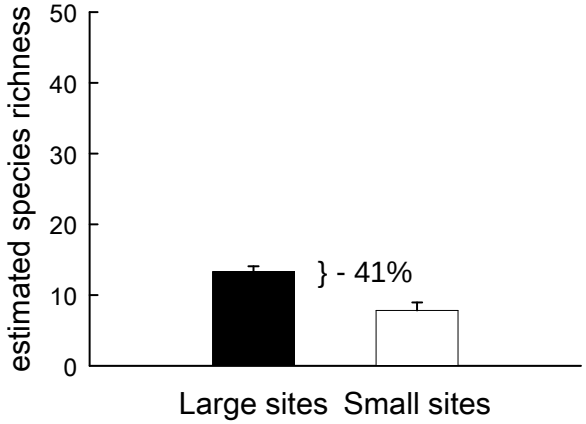
a) Butterfly specialists



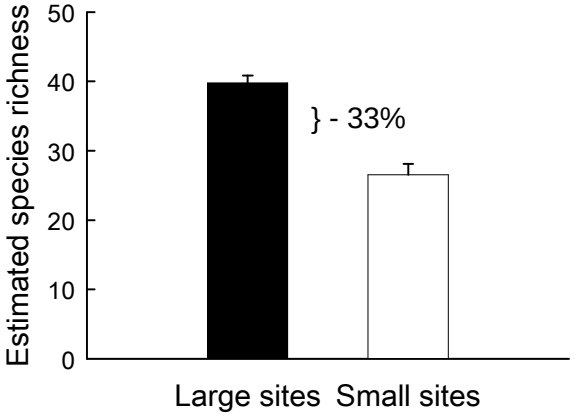
b) Butterfly generalists



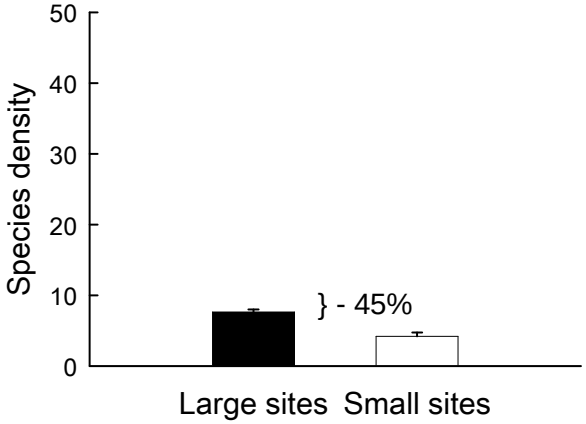
c) Butterfly specialists



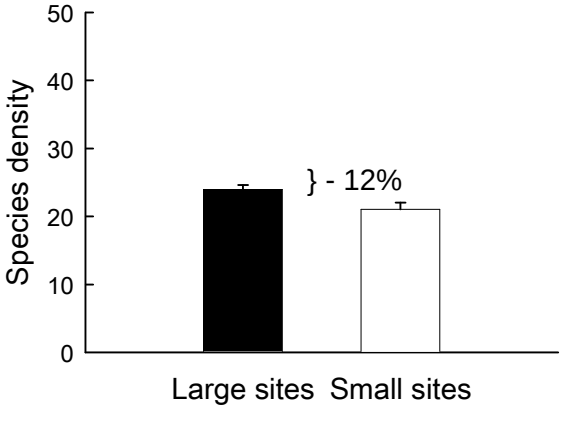
d) Butterfly generalists



e) Butterfly specialists



f) Butterfly generalists



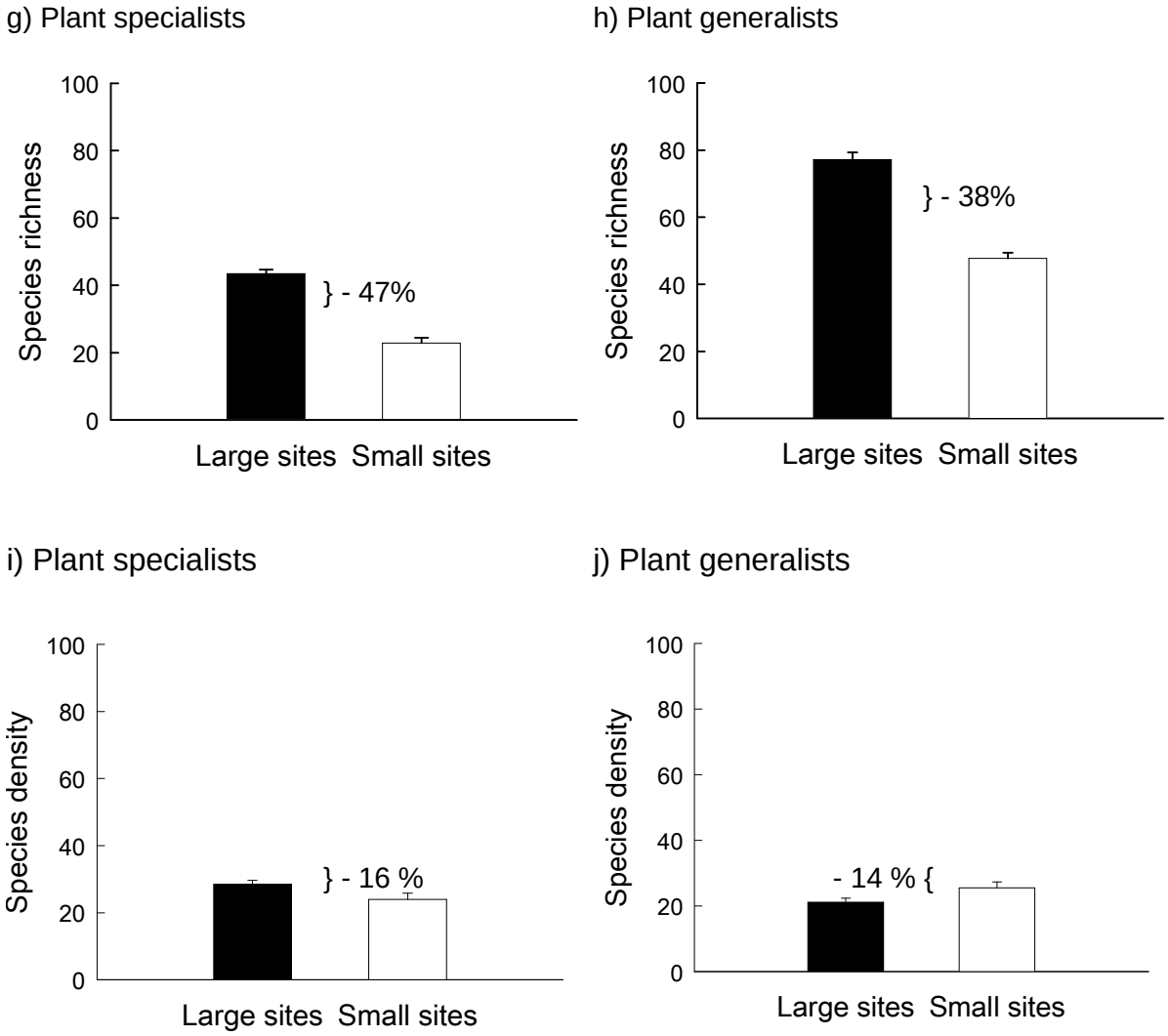


Fig. 3 Species richness, estimated species richness (only for butterflies), and species density of butterfly and plant specialists and generalists in large (black) versus small (white) study sites.
Species richness: a) butterfly specialists, b) butterfly generalists;
Estimated species richness: c) butterfly specialists, d) butterfly generalists;
Species density: e) butterfly specialists, f) butterfly generalists;
Species richness: g) plant specialists, h) plant generalists;
Species density: i) plant specialists, j) plant generalists.
All figures show significantly different means (statistics see Table 1).

NMDS ordinations confirmed our results and showed that large patches and small patches differed in community patterns. Thereby small patches were more dissimilar than large patches and the patterns were clearer for butterflies than for

plants. For community composition the habitat area (small vs. large sites) was more relevant than connectivity (Fig. S3, page 79).

Discussion

It is important to know which local and landscape factors are necessary for the conservation of habitat specialist species. For example, the arrangement and size of patches might be important for species persistence. It has been suggested that habitat quality and area are relevant, as well as connectivity and landscape context (e.g. Dennis & Eales 1999; Stefanescu et al. 2004; Dover & Settele 2009). Our study makes three relevant contributions: first, we show that habitat connectivity is of particular importance for specialized butterfly and plant species. Secondly, a comparison of different connectivity measures reveals that an index combining neighboring patch area and distance is more suitable than using cover of habitat or distance to the next habitat. Thirdly, we find clear differences in the species richness, species composition and proportion of habitat specialists in small versus large habitat patches suggesting that small patches have already lost most butterfly and plant specialists.

Effects of connectivity

Connectivity of habitats is an important determinant of species richness and abundance of specialized species in our study. These findings are in contrast to previous studies on plant and butterfly species richness (Wilcox et al. 1986; Steffan-Dewenter & Tscharntke 2000; Krauss et al. 2003; Krauss et al. 2004; Bisteau & Mahy 2005). This might be due to the focus of these studies on species-area relationships, to intercorrelations between area and connectivity, or to a partial coverage of regional connectivity gradients. Other studies showed marginally significant effects of connectivity on plant species richness (Adriaens et al. 2006), or an impact of historical landscape connectivity on present day plant diversity (Lindborg & Eriksson 2004), indicating a slow response of plant species richness to fragmentation (Helm et al. 2006). Thus, delayed extinction of local plant populations might partly mask the relevance of habitat connectivity for long-living species like plants (Kuussaari et al. 2009). Indeed, we found more severe species loss for butterflies than for plants with decreasing connectivity.

This might also be caused by different dispersal mechanisms between plants and butterflies. Seed dispersal to new habitats takes place passively by wind or animal transport but this might be lacking if calcareous grasslands are not regularly grazed by sheep (Wessels et al. 2008). In contrast butterflies are active dispersers, have a visual orientation and can choose where to fly (Hambäck et al. 2007).

Even though small sites might not be able to maintain viable populations and thus rely more on immigration from surrounding habitat patches than large sites (Öckinger & Smith 2006), we found no differences between large and small study sites in species-connectivity relationships (no significant interactions). The generally strong connectivity effects in our data underline the importance of immigration events for the long-term survival of species with specialized habitat requirements not only in small but also in relatively larger patches.

Connectivity measures

We found strong effects of Hanski's Connectivity Index on species richness and species density of specialized butterflies and plants. Percentage habitat cover was also a suitable connectivity measure for specialized plant species density, whereas distance to the next habitat patch was generally a weak predictor. In our landscapes many small habitat patches were scattered around the focal study sites so that distances to the next habitat patch were often small, and furthermore these small patches might not represent sources for immigration (Boughton 1999). Therefore, it is reasonable that a connectivity index which combines distance and patch area of neighboring habitats is more suitable to quantify differences in habitat connectivity. However, for extremely fragmented landscapes or landscapes with a clear mainland-island situation the Euclidian distance to next habitat patch can be useful and has been successfully applied (Winfree et al. 2005). Habitat cover is a good connectivity measure for situations with large proportions of habitats nearby (Winfree et al. 2005). In a study in the Swiss Alps habitat cover ranged from 0 to 27% on landscape scales between 500 – 4000 m radii, and was a good predictor for butterfly species occurrence (Cozzi et al. 2008). However habitat cover includes neither the distances from the focal study sites to all the habitat patches in the landscape, nor a scaling of size for the adjacent habitat

patches, giving all habitats within a certain radius the same weight (Moilanen & Nieminen 2002). In our study region with a habitat cover gradient from 0.01 to 2.2%, Hanski's Connectivity Index was the best predictor. Further connectivity measures exist, taking functional responses of species into account, like dispersal ability and the probability of dispersing between habitat patches (Kindlmann & Burel 2008) or the spatial arrangement of habitat patches in the surrounding landscape (Matisziw & Murray 2009). These new indices will definitely contribute to connectivity research for single species studies. However for community studies we suggest the use of somewhat simpler connectivity measures depending on patch configuration, landscape context and study design.

Specialists and generalists

In contrast to habitat specialists, there was little impact of connectivity on butterfly and plant species which are not specialized on calcareous grasslands. For reasons of simplicity we call these species "generalists". As generalists can occur in the matrix, an effect of connectivity was not expected.

Habitat area was a strong predictor for habitat specialist but also generalist species. However specialists lose a higher proportion of species, when habitat area is lost. This might be explained by edge-effects where generalists profit from a higher proportion of habitat edges when habitat patch size is decreasing (Laurance & Yensen 1991), whereas edges are less-preferred by habitat specialists (Ries & Sisk 2008). Habitat specialist butterflies have been shown to suffer more from habitat loss than generalists (Warren et al. 2001; Krauss et al. 2003). The seeds of habitat specialist plant species need specific conditions to survive. For example an influx of nitrogen from habitat edges might decrease species richness of specialized plants, whereas generalist species might survive better at edges and consequently show a higher dominance in small habitat patches (Jacquemyn et al. 2003). Such edge effects might explain why generalist plants in our study showed higher species densities and abundances in small compared to large patches.

Habitat area and quality

The species richness of both specialists and generalists depended on habitat area, which confirms the general validity of the species-area relationship (e.g.

Wilcox et al. 1986; Rosenzweig 1995; Wettstein & Schmid 1999; Bruun 2000; Zschokke et al. 2000; Krauss et al. 2009). The positive relationship between the abundance of butterflies and increasing habitat area has also been shown before (e.g. Krauss et al. 2003). However our results show that specialist species density (equal sample size) is higher on large compared to small patches. Sampling effort should therefore play a minor role, giving more importance to “area per se” and “habitat heterogenity” explanations for our species-area relationship (Ouin et al. 2006; Krauss et al. 2009). Species density-area relationships are less frequently studied than species-area relationships (but see e.g. Wettstein & Schmid 1999; Krauss et al. 2004; Lindborg & Eriksson 2004).

NMDS ordination confirmed the finding that large sites differ from small sites in their community composition and that habitat area played a major role. Butterfly communities differed clearly between large and small sites whereas this difference was less distinct for plant species as smaller sites exhibited very heterogeneous species composition.

Habitat quality factors did not have a significant effect on plant and butterfly species richness, but the study design was chosen to keep quality between study sites as similar as possible. However large and small sites did differ in habitat quality and at least some of the variance explained by the area of small versus large sites could be related to differences in habitat quality. Habitat quality was not the main focus of this study, even though its importance has been often highlighted (e.g. Dennis & Eales 1999; Adriaens et al. 2006; Kuussaari et al. 2007; Raatikainen et al. 2007). We also found no effect of management (grazed/mown vs. fallows) on species richness. Similarly in Swiss grasslands, butterfly species diversity did not differ between early fallows (no management for 2-3 years) and managed calcareous pastures (Balmer & Erhardt 2000). Unmanaged grasslands in our region were similar to early fallows. In addition, as our study focused on the community level and not on single species, habitat quality for species communities is difficult to define and might be of less overall importance due to variability in habitat requirements.

Conclusions

Habitat specialist butterfly and plant species were highly dependent on connectivity and habitat area. The best connectivity measure in our study region was a connectivity index, which takes into account the area of all habitat patches with their distances to the focal habitat. In contrast, (i) distance to the next habitat patch and the slightly better (ii) amount of habitat in a landscape were both unsuitable connectivity indicators for our study region. We suggest that distance to the next habitat might be a good connectivity measure for distinct mainland-island situations and in highly fragmented landscapes with few remaining habitat patches, which contain viable populations (source habitats). The proportion of habitat within a landscape of a specific radius is a reasonable measure, if the proportion of habitat is relatively high. However for most landscapes with intermediate to high fragmentation levels Hanski's Connectivity Index is probably the best approach to detect connectivity effects on species richness (Winfree et al. 2005; Cozzi et al. 2008). We found that if all habitat patches in our landscape were lost except for the focal study site, there would be a loss of 38-69% of specialized butterfly species and 24-37% of specialized plant species. Thus, disruption of habitat connectivity would lead to significant future extinctions of species in addition to extinctions due to habitat loss per se. Conservation management should therefore seek to improve connectivity at a landscape scale in addition to the protection and adequate management of conservation areas at a patch scale. We recommend conserving large grasslands and suggest regular grazing or mowing of these to keep the habitat quality suitable for butterfly and plant species that are specialized on calcareous grasslands. We also encourage active restoration of patches that have once been calcareous grasslands (e.g. by removing trees and bushes) in order to increase habitat connectivity. This will be particularly important for mitigation of possible extinction debt and the long-term survival of habitat specialists in highly fragmented semi-natural grasslands in Europe.

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Appendix

Table S1 Mean, standard error, Min and Max of local habitat quality factors: patch size (ha), patch perimeter (m), flower cover (%), bush cover (%).

		Mean	SE	Min	Max
large sites	patch size	2.41	0.24	0.83	5.80
	patch perimeter	1157.75	87.92	547.57	2340.13
	flower cover	18.85	0.86	11.13	29.38
	bush cover	19.35	1.79	5	40
small sites	patch size	0.12	0.02	0.01	0.35
	patch perimeter	232.36	16.82	69.60	467.25
	flower cover	13.84	0.84	4.00	22.50
	bush cover	34.35	3.76	5	70

Table S2 Pearson correlation coefficients (*r*) among the predictor variables of the 31 study sites of the large and the small sites (*** p < 0.001; * p < 0.05; (*) p < 0.1; ns = not significant; CI = Connectivity Index; distance = distance to next suitable patch; habitat cover = % of calcareous grassland cover; habitat area = small or large study site.

		habitat cover	distance	CI	manage-ment	bush cover	flower cover
large sites	patch size	-0.22 ns	-0.002 ns	-0.06 ns	0.11ns	0.11 ns	-0.34 (*)
	habitat cover		-0.31 (*)	0.75 ***	0.27 ns	0.01 ns	0.04 ns
	distance			-0.44 *	-0.15 ns	-0.12 ns	0.35 (*)
	CI				0.18 ns	-0.06 ns	-0.13 ns
	management					-0.26 ns	-0.11 ns
	bush cover						-0.01 ns
small sites	patch size	-0.05 ns	-0.19 ns	0.06 ns		-0.19 ns	-0.20 ns
	habitat cover		-0.24 ns	0.76 ***		0.25 ns	0.25 ns
	distance			-0.41 *		0.02 ns	-0.04 ns
	CI					0.06 ns	0.17 ns
	bush cover						-0.14 ns

Table S3 Single factor analyses for butterfly and plant specialist and generalist species richness with the predictor variables of habitat quality. All relations are not significant.

		Single factor analyses						
		$F_{1,29}$	**p**	**r**		**$F_{1,29}$**	**p**	**r**
large sites	**butterfly specialists**				**butterfly generalists**			
	patch size	1.69	0.20	-0.24	patch size	3.85	0.06	-0.34
	flower cover	2.42	0.13	0.28	flower cover	0.58	0.45	0.14
	bush cover	0.72	0.40	0.16	bush cover	0.05	0.83	-0.04
	management	0.18	0.67	-0.24	management	0.39	0.54	-0.02
	plant specialists				**plant generalists**			
	patch size	0.69	0.41	-0.15	patch size	1.94	0.18	0.25
	bush cover	0.05	0.82	-0.04	bush cover	3.89	0.06	-0.34
	management	0.26	0.61	-0.10	management	0.08	0.78	-0.03
small sites	**butterfly specialists**				**butterfly generalists**			
	patch size	0.84	0.37	0.17	patch size	0.51	0.48	0.13
	flower cover	2.58	0.12	0.29	flower cover	1.25	0.27	0.20
	bush cover	0.01	0.94	-0.01	bush cover	0.01	0.93	-0.02
	plant specialists				**plant generalists**			
	patch size	0.30	0.59	-0.10	patch size	3.35	0.08	-0.32
	bush cover	0.53	0.47	-0.13	bush cover	1.07	0.31	0.19

Table S4 Species identified in 2007 on 62 calcareous grasslands in the "Fränkische Schweiz" (Bavaria, Germany). a) plant species, b) butterfly species (including burnet moths); G = classified as generalist species, S = classified as habitat specialist species.

a) Plant species

Plant species	specialization	Plant species	specialization
Acer campestre	G	Lathyrus spec.	G
Acer platanoides	G	Lathyrus sylvestris	G
Acer pseudoplatanus	G	Lathyrus tuberosus	G
Achillea millefolium agg.	G	Leontodon autumnalis	G
Acinos arvensis	S	Leontodon hispidus	G
Aegopodium podagraria	G	Lepidium campestre	G
Agrimonia eupatoria	G	Leucanthemum vulgare	S
Agrostis capillaris	G	Ligustrum vulgare	G
Agrostis gigantea	G	Linaria vulgaris	G
Ajuga genevensis	S	Linum austriacum	S
Ajuga pyramidalis	G	Linum catharticum	S
Ajuga reptans	G	Listera ovata	G
Alchemilla spec.	G	Lithospermum officinale	G
Alchemilla vulgaris	G	Lolium perenne	G
Alliaria petiolata	G	Lonicera xylosteum	G
Allium oleraceum	G	Lotus corniculatus	G
Allium scorodoprasum	G	Luzula campestris	G
Allium senescens	S	Lysmachia punctata	G
Allium vineale	S	Malva alcea	G
Alnus incana	G	Matricaria recutita	G
Alopecurus pratensis	G	Medicago falcata	S
Alyssum alyssoides	S	Medicago lupulina	G
Anemone nemorosa	G	Medicago minima	S
Anemone sylvestris	S	Medicago x varia	G
Angelica sylvestris	G	Melampyrum arvense	S
Antennaria dioica	S	Melampyrum cristatum	G
Anthemis tinctoria	S	Melampyrum pratense	G
Anthericum ramosum	S	Melampyrum sylvaticum	G
Anthoxanthum odoratum	G	Melica nutans	G
Anthriscus sylvestris	G	Melilotus officinalis	G
Anthyllis vulneraria	S	Melilotus spec.	G
Aquilegia vulgaris	G	Mycelis muralis	G
Arabidopsis thaliana	S	Myosotis arvensis	G
Arabis hirsuta	S	Odontites vernus	G

Arenaria serpyllifolia	S	Onobrychis viciifolia	S
Arrhenatherum elatius	G	Ononis repens	S
Artemisia absinthium	G	Ononis spec.	G
Artemisia campestris	S	Ononis spinosa	G
Artemisia spec.	G	Ophrys insectifera	S
Artemisia vulgaris	G	Orchis militaris	S
Asperula cynanchica	S	Orchis purpurea	G
Asplenium ruta-muraria	G	Origanum vulgare	S
Asplenium trichomanes	G	Papaver rhoeas	G
Aster amellus	S	Pastinaca sativa	G
Astragalus glycyphyllos	G	Petrorhagia prolifera	S
Avena sativa	G	Peucedanum cervaria	S
Avenula pubescens	G	Phleum phleoides	S
Bellis perennis	G	Phleum pratense	G
Berberis vulgaris	G	Picea abies	G
Betonica officinalis	G	Picris hieracioides	G
Betula pendula	G	Pimpinella saxifraga	S
Brachypodium pinnatum	S	Pinus sylvestris	G
Brachypodium sylvaticum	G	Plantago lanceolata	G
Briza media	S	Plantago major	G
Bromus arvensis	G	Plantago media	G
Bromus erectus	S	Poa annua	G
Bromus hordeaceus	G	Poa compressa	G
Bromus tectorum	G	Poa nemoralis	G
Buphthalmum salicifolium	S	Poa pratensis	G
Bupleurum falcatum	G	Poa trivialis	G
Calamagrostis varia	G	Polygala amarella	G
Calluna vulgaris	G	Polygala chamaebuxus	S
Calystegia sepium	G	Polygala comosa	S
Campanula patula	G	Polygala vulgaris	G
Campanula persicifolia	G	Polygonatum odoratum	S
Campanula rapunculoides	G	Populus nigra	G
Campanula rotundifolia	G	Populus tremula	G
Campanula trachelium	G	Potentilla anserina	G
Capsella bursa-pastoris	G	Potentilla argentea	S
Cardamine pratensis	G	Potentilla erecta	G
Cardaminopsis petraea	S	Potentilla neumanniana	S
Carduus acanthoides	G	Potentilla recta	G
Carduus crispus	G	Potentilla reptans	G
Carduus nutans	G	Primula veris	S
Carex caryophyllea	S	Prunella grandiflora	S

Carex digitata	G	Prunus avium	G
Carex flacca	G	Prunus fruticosa	G
Carex hirta	G	Prunus mahaleb	G
Carex montana	S	Prunus serotina	G
Carex muricata agg.	G	Prunus spinosa	G
Carex sylvatica	G	Pteridium aquilinum	G
Carlina acaulis	S	Pulsatilla vulgaris	S
Carlina vulgaris	S	Pyrus pyraster	G
Carpinus betulus	G	Quercus petraea	G
Carum carvi	G	Quercus robur	G
Centaurea jacea	G	Ranunculus acris	G
Centaurea scabiosa	S	Ranunculus bulbosus	G
Cerastium arvense	G	Ranunculus repens	G
Cerastium holosteoides	G	Rhamnus cathartica	G
Chaerophyllum hirsutum	G	Rhinanthus alectorolophus	G
Chaerophyllum temulum	G	Rhinanthus angustifolius	S
Chelidonium majus	G	Rhinanthus glacialis	S
Cichorium intybus	G	Rhinanthus serotinus	S
Cirsium acaule	S	Ribes spec.	G
Cirsium arvense	G	Ribes uva-crispa	G
Cirsium eriophorum	S	Robinia pseudoacacia	G
Cirsium oleraceum	G	Rosa canina	G
Cirsium spec.	G	Rosa rubiginosa	G
Cirsium vulgare	G	Rosa spec.	G
Clematis vitalba	G	Rubus caesius	G
Clinopodium vulgare	S	Rubus fruticosus agg.	G
Colchicum autumnale	G	Rubus idaeus	G
Convallaria majalis	G	Rubus spec.	G
Convolvulus arvensis	G	Rumex acetosa	G
Cornus sanguinea	G	Rumex acetosella	S
Coronilla varia	S	Salix caprea	G
Corylus avellana	G	Salvia pratensis	G
Cotoneaster integerrimus	S	Sambucus nigra	G
Crataegus laevigata	G	Sanguisorba minor	S
Crataegus monogyna	G	Saponaria officinalis	G
Crepis biennis	G	Saxifraga granulata	G
Crocus spec.	G	Scabiosa columbaria	S
Cruciata laevipes	G	Scirpus sylvaticus	G
Cynoglossum officinale	G	Scrophularia nodosa	G
Cynosurus cristatus	G	Sedum acre	S
Dactylis glomerata	G	Sedum album	S

Daucus carota	G	Sedum forsterianum	G
Deschampsia cespitosa	G	Sedum maximum	G
Dianthus carthusianorum	S	Sedum sexangulare	S
Dipsacus fullonum	G	Sedum telephium agg.	G
Draba aizoides	S	Senecio erucifolius	G
Dryopteris filix-mas	G	Senecio jacobaea	G
Echium vulgare	S	Senecio spec.	G
Elytrigia repens	G	Senecio vulgaris	G
Epilobium angustifolium	G	Seseli annuum	S
Epilobium roseum	G	Sesleria albicans	G
Epipactis atrorubens	G	Silaum silaus	G
Equisetum arvense	G	Silene dioica	G
Erigeron acris	G	Silene latifolia	G
Erigeron annuus	S	Silene nutans	G
Erodium cicutarium	G	Silene vulgaris	G
Eryngium campestre	S	Solidago canadensis	G
Erysimum crepidifolium	S	Solidago spec.	G
Euonymus europaea	G	Solidago virgaurea	G
Euphorbia cyparissias	S	Sonchus arvensis	G
Euphrasia officinalis	G	Sonchus asper	G
Fagus sylvatica	G	Sonchus oleraceus	G
Festuca ovina agg.	S	Sonchus spec.	G
Festuca pratensis	G	Sorbus aria	G
Festuca rubra	G	Sorbus aucuparia	G
Filipendula ulmaria	G	Sorbus spec.	G
Fragaria viridis	S	Sorbus torminalis	G
Frangula alnus	G	Stachys recta	S
Fraxinus excelsior	G	Stachys sylvatica	G
Galeopsis pubescens	G	Stellaria graminea	G
Galeopsis tetrahit	G	Stellaria holostea	G
Galium album	G	Stellaria nemorum	G
Galium aparine	G	Tanacetum corymbosum	G
Galium boreale	S	Tanacetum vulgare	G
Galium mollugo	G	Taraxacum laevigatum	S
Galium pumilum	S	Taraxacum officinale	G
Galium spurium	G	Taraxacum sect. Erythrosperma	G
Galium verum	S	Taraxacum sect. Ruderalia	G
Genista tinctoria	S	Taraxacum spec.	G
Gentiana cruciata	S	Teucrium botrys	S
Gentianella ciliata	S	Teucrium chamaedrys	S

Geranium columbinum	G	Thlaspi perfoliatum	G
Geranium molle	G	Thymus pulegioides	S
Geranium pratense	G	Tilia cordata	G
Geranium purpureum	G	Tilia platyphyllos	G
Geranium pusillum	G	Torilis japonica	G
Geranium robertianum	G	Tragopogon pratensis	G
Geranium sanguineum	S	Trifolium arvense	S
Geum urbanum	G	Trifolium campestre	G
Glechoma hederacea	G	Trifolium dubium	G
Globularia bisnagarica	S	Trifolium medium	G
Gymnadenia conopsea	S	Trifolium montanum	S
Hedera helix	G	Trifolium pratense	G
Helianthemum nummularium	S	Trifolium repens	G
Helictotrichon parlatorei	G	Tripleurospermum perforatum	G
Helictotrichon pratense	S	Trisetum flavescens	G
Helictotrichon pubescens	G	Tussilago farfara	G
Heracleum sphondylium	G	Urtica dioica	G
Hieracium bifidum	S	Valeriana officinalis	G
Hieracium lachenalii	G	Valerianella locusta	G
Hieracium maculatum	G	Verbascum densiflorum	G
Hieracium murorum	G	Verbascum lychnitis	G
Hieracium pilosella	S	Verbascum nigrum	G
Hieracium piloselloides	S	Verbascum spec.	G
Hieracium spec.	G	Verbascum thapsus	G
Hieracium umbellatum	G	Verbena officinalis	G
Hippocrepis comosa	S	Veronica arvensis	G
Holcus lanatus	G	Veronica chamaedrys	G
Hypericum hirsutum	G	Veronica hederifolia	G
Hypericum humifusum	G	Veronica officinalis	G
Hypericum maculatum	S	Veronica serpyllifolia	G
Hypericum perforatum	G	Veronica teucrium	S
Hypochaeris maculata	S	Viburnum lantana	G
Hypochaeris radicata	G	Viburnum opulus	G
Inula conyzae	S	Vicia angustifolia	G
Inula salicina	S	Vicia cracca	G
Iris germanica	G	Vicia grandiflora	G
Juglans regia	G	Vicia hirsuta	G
Juniperus communis	G	Vicia sepium	G
Knautia arvensis	G	Vicia spec.	G
Koeleria pyramidata	S	Vicia tenuifolia	G
Lactuca serriola	G	Vincetoxicum hirundinaria	S

Lamium album	G	Viola arvensis	G
Lamium maculatum	G	Viola canina	G
Lamium purpureum	G	Viola collina	G
Lathyrus latifolius	G	Viola hirta	G
Lathyrus nissolia	G	Viola odorata	G
Lathyrus odoratus	G	Viola spec.	G
Lathyrus pratensis	G	Vitis vinifera	G

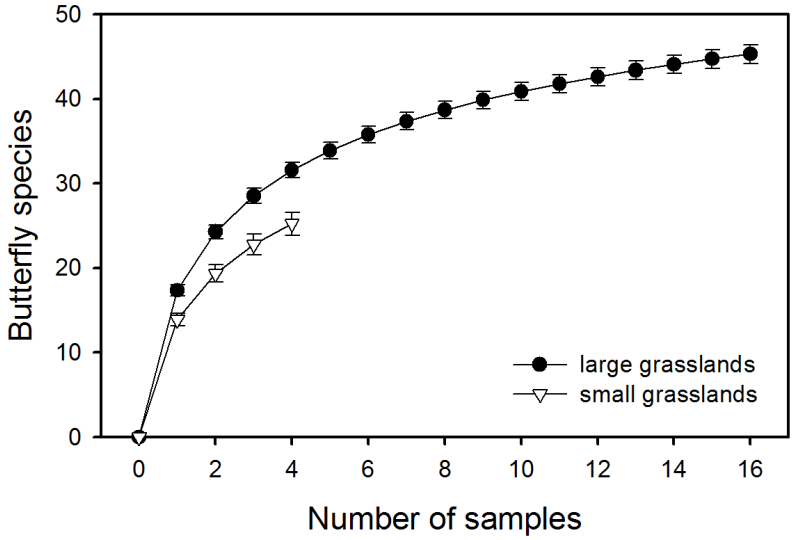
b) Butterfly species

Butterfly species	**specialization**	**Butterfly species**	**specialization**
Adscita geryon	S	Melitaea phoebe	S
Adscita statices	G	Nymphalis antiopa	G
Anthocharis cardamines	G	Nymphalis c-album	G
Apatura ilia	G	Nymphalis io	G
Aphantopus hyperantus	G	Nymphalis urticae	G
Aporia crataegi	G	Ochlodes sylvanus	G
Araschnia levana	G	Papilio machaon	G
Argynnis adippe	G	Parargе aegeria	G
Argynnis aglaja	G	Pieris brassicae	G
Argynnis paphia	G	Pieris napi	G
Boloria dia	S	Pieris rapae	G
Boloria euphrosyne	G	Plebeius argus	G
Brenthis ino	G	Plebeius argyrognomon	S
Callophrys rubi	G	Plebeius idas	G
Carterocephalus palaemon	G	Polyommatus agestis	S
Celastrina argiolus	G	Polyommatus amandus	G
Coenonympha arcania	G	Polyommatus bellargus	S
Coenonympha glycerion	G	Polyommatus coridon	S
Coenonympha pamphilus	G	Polyommatus daphnis	G
Colias alfacariensis/hyale	S	Polyommatus dorylas	S
Cupido minimus	S	Polyommatus icarus	G
Erebia aethiops	G	Polyommatus semiargus	G
Erebia medusa	G	Polyommatus thersites	S
Erynnis tages	G	Pyrgus trebeviciensis	S
Gonepteryx rhamni	G	Pyrgus malvae	G
Hamearis lucina	G	Pyrgus serratulae	S
Hesperia comma	S	Satyrium acaciae	S
Issoria lathonia	G	Satyrium spini	S
Jordanita globulariae	S	Spialia sertorius	S
Jordanita notata	S	Thecla betulae	G

Lasiommata megera	G	Thymelicus acteon	S
Leptidea sinapis/reali	G	Thymelicus lineola	G
Limenitis camilla	G	Thymelicus sylvestris	G
Limenitis populi	G	Vanessa atalanta	G
Lycaena hippothoe	G	Vanessa cardui	G
Lycaena phlaeas	G	Zygaena angelicae	G
Lycaena tityrus	G	Zygaena carniolica	S
Maculinea arion	S	Zygaena ephialtes	G
Maniola jurtina	G	Zygaena filipendulae	G
Melanargia galathea	G	Zygaena lonicerae	S
Melitaea athalia	G	Zygaena loti	G
Melitaea aurelia	S	Zygaena minos	S
Melitaea britomartis	S	Zygaena purpuralis	S
Melitaea cinxia	G	Zygaena viciae	G
Melitaea diamina	G		

Fig. S1 Species accumulation curves for butterfly species a), and plant species b) in the year 2007 with number of samples: large grasslands (n = 31), and small grasslands (n = 31). Each sample are data of a pooled time period (5 transect minutes of all eight transect walks). Shown are arithmetic means with SEs. Data were smoothed with 1000 times randomisation with the program EstimateS, version 8 (Colwell 2006). Study site saturation values (SOB/ACE) ranged from 75% to 97% for butterfly species from large sites and from 58% to 95% from small sites. Values for SOB/ACE ranged from 85% to 99% for plant species from large sites and from 78% to 97% for small sites.

a)



b)

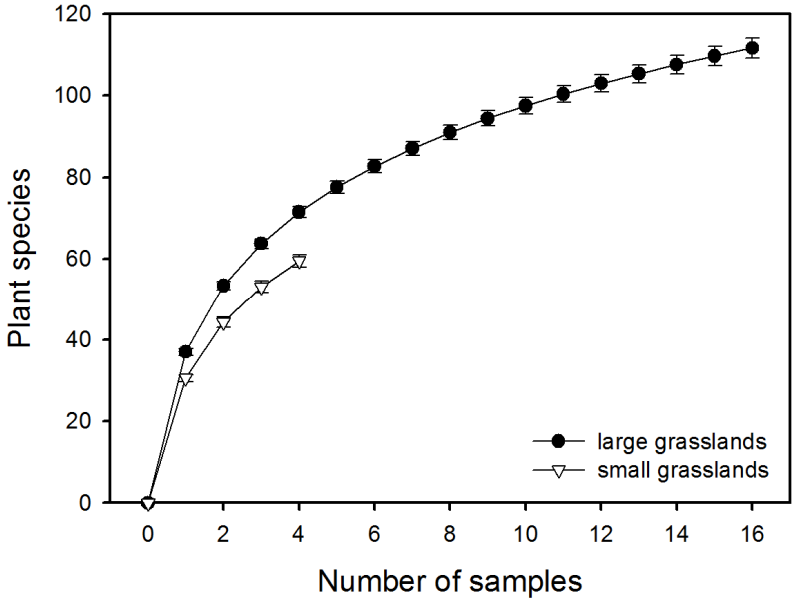
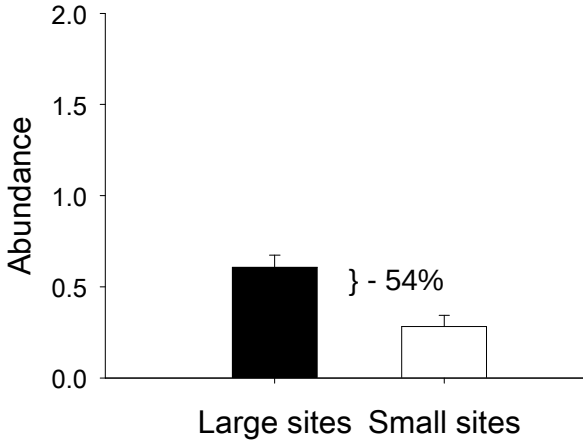
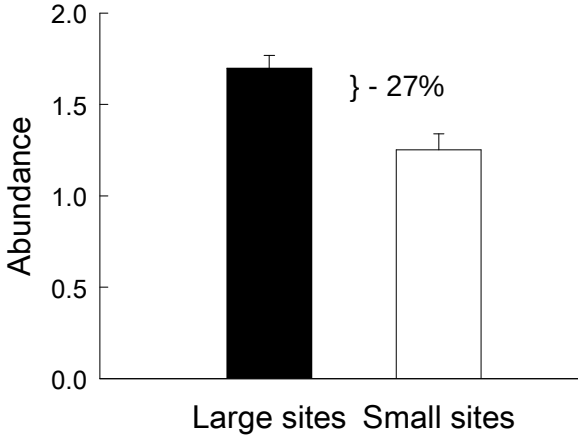


Fig. S2 Abundances of butterfly and plant specialists and generalists in large (black) versus small (white) study sites, abundance of plants is in m^2, abundance of butterflies is per 100 m^2. a) butterfly specialists, b) butterfly generalists, c) plant specialists, d) plant generalists.

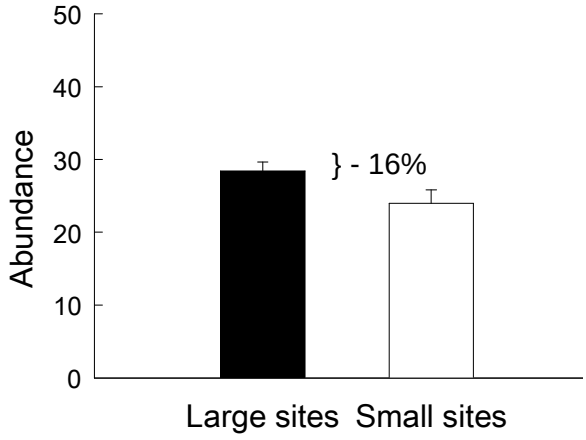
a) Butterfly specialists



b) Butterfly generalists



c) Plant specialists



d) Plant generalists

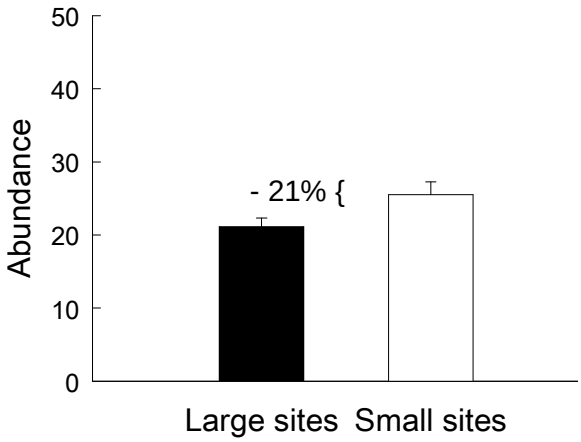


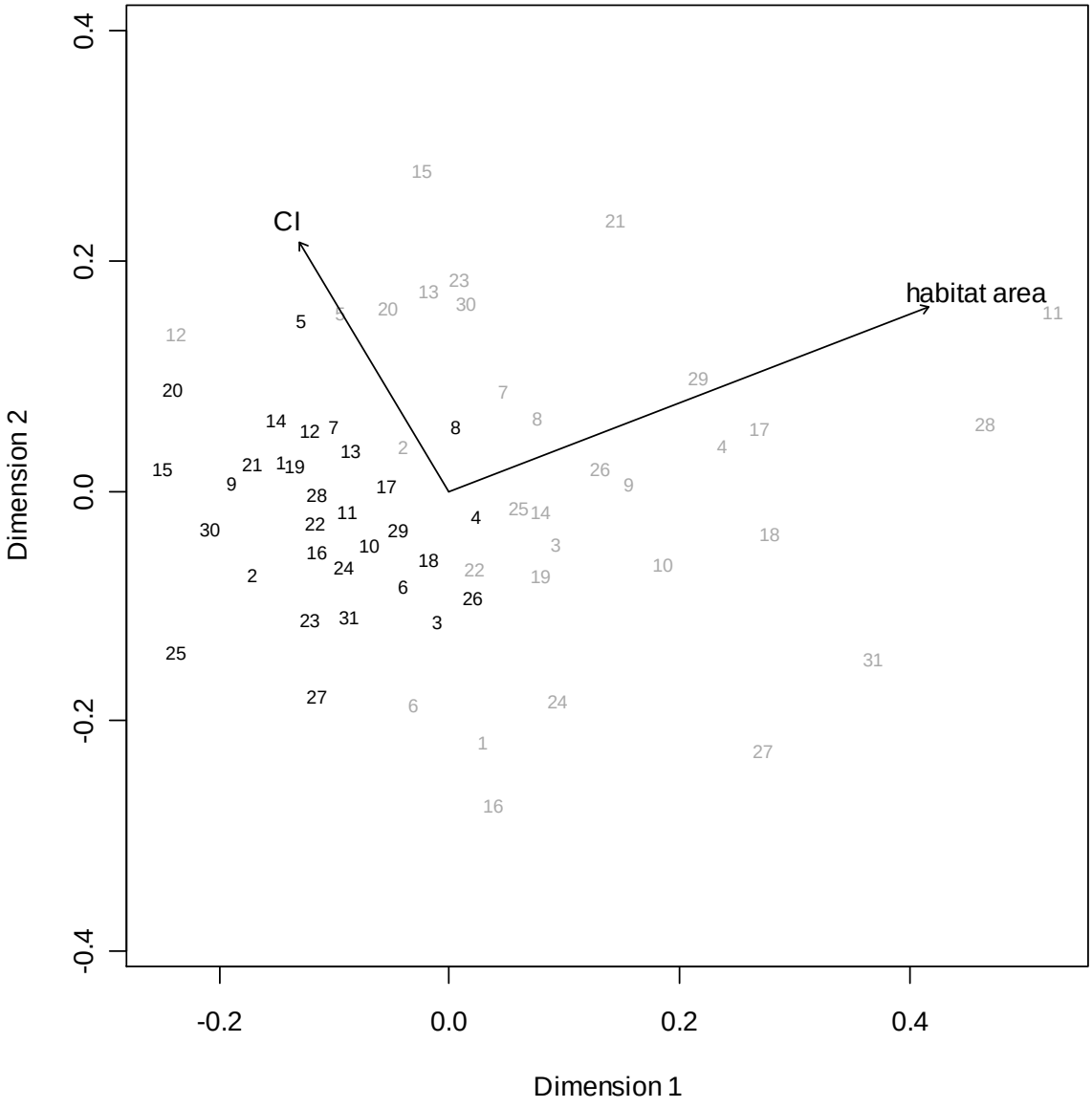
Fig. S3 Nonmetric Multidimensional Scaling (NMDS)

a) butterfly occurrence including standardized abundances STRESS = 22.34%,

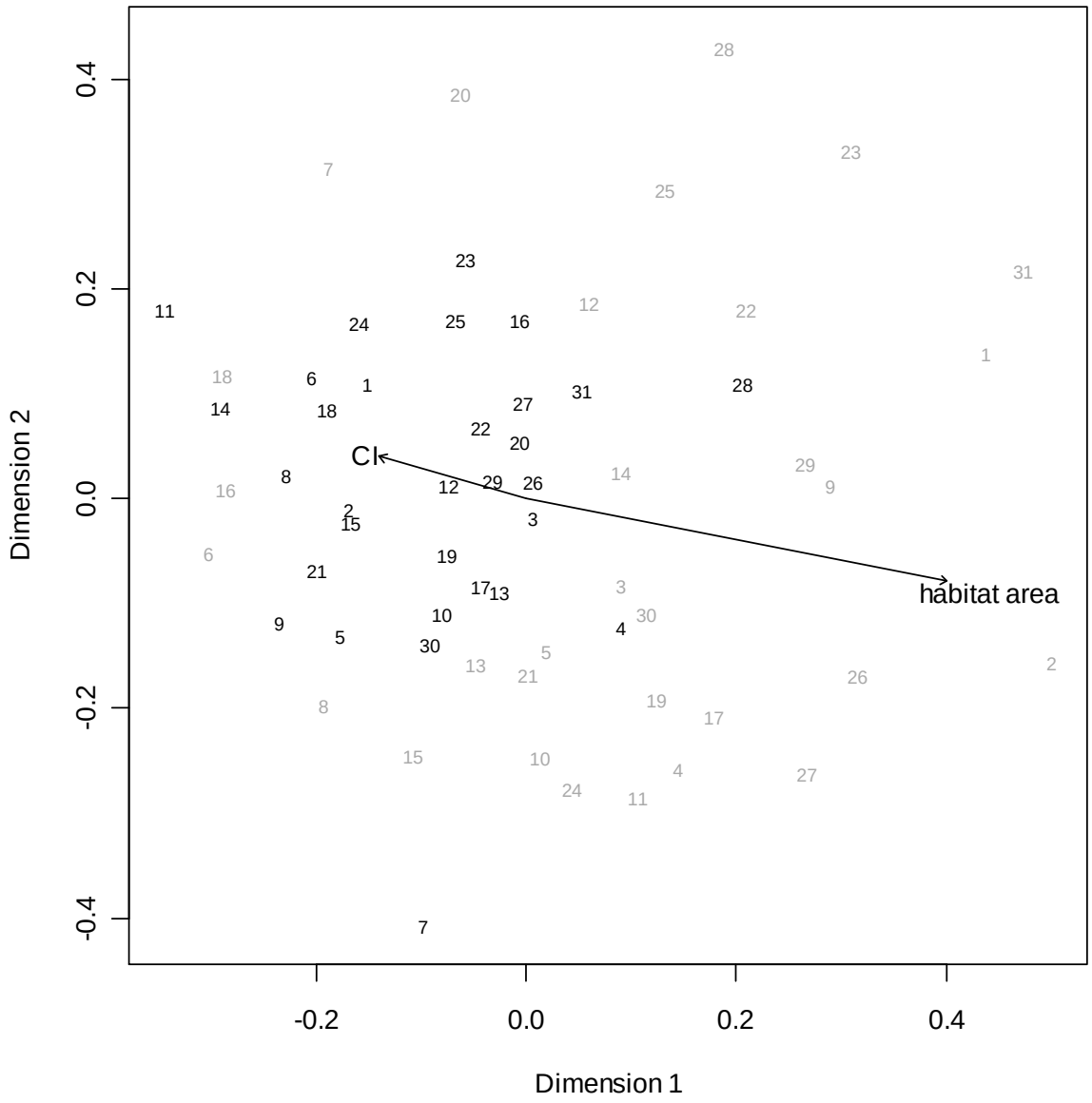
b) plant occurrence including standardized abundances, STRESS = 23.21%.

Black numbers = large sites (1-31), grey numbers = small sites (1-31).

a)



b)


Dimension 2
0.4
0.2
0.0
-0.2
-0.4
Cl
habitat area
-0.2
0.0
0.2
0.4
Dimension 1

6. Impact of habitat fragmentation on trophic interactions

The impact of habitat fragmentation on trophic interactions of the monophagous butterfly *Polyommatus coridon*

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Abstract

Theory predicts that habitat fragmentation, including reduced area and connectivity of suitable habitat, changes multitrophic interactions. Species at the bottom of trophic cascades (host plants) are expected to be less negatively affected than higher trophic levels, such as herbivores and their parasitoids or predators. Here we test this hypothesis regarding the effects of habitat area and connectivity in a trophic system with three levels: first with the population size of the larval food plant *Hippocrepis comosa*, next with the population density of the monophagous butterfly species *Polyommatus coridon* and finally with its larval parasitism rate. Our results show no evidence for negative effects of habitat fragmentation on the food plant or on parasitism rates, but population density of adult *P. coridon* was reduced with decreasing connectivity. We conclude that the highly specialized butterfly species is more affected by habitat fragmentation than its larval food plant because of its higher trophic position. However, the butterfly host species was also more affected than its parasitoids, presumably because of lower resource specialization of local parasitoids which also frequently occur in alternative hosts. Therefore conservation efforts should focus first on the most specialized species of interaction networks and second on higher trophic levels.

Introduction

Habitat fragmentation, including habitat loss and reduced habitat connectivity, is known to have negative impacts on local, regional and global biodiversity (Saunders et al. 1991; Debinski & Holt 2000; Ewers & Didham 2006). Highly fragmented calcareous grasslands rank as the most species-rich habitat of flora and invertebrate fauna in central Europe (Poschlod & Wallis De Vries 2002). Plant and invertebrate species restricted to these semi-natural habitats are threatened because of decreasing habitat area, connectivity and habitat quality consequent to agricultural intensification and the abandonment of historical land-use practices (Dennis & Eales 1997; Pärtel et al. 1999; Dauber et al. 2006; Brereton et al. 2008; Krauss et al. 2010).

Habitat area is one of the main factors determining species occurrences. Small patches often maintain no viable populations and depend on immigration from nearby habitats (Hanski et al. 1996). Small habitat patches are more often of poor

habitat quality than large habitat patches, thus leading to further diversity declines (Fahrig 2003; Dobson et al. 2006). As habitat area decreases, so does the connectivity between patches, leading to larger distances between habitat patches. Lack of connectivity to large source habitats limits the exchange of individuals and prevents re-colonization events (Baguette et al. 2000). Species at higher trophic levels (herbivores, predators, parasitoids) are expected to be even more affected than lower trophic levels, as they depend on their resources (host species) and additionally, directly or indirectly, on habitat area and connectivity. Furthermore, higher trophic levels perceive habitats, with their patchily distributed specific resources, as more fragmented than do lower trophic levels (van Nouhuys 2005). Hereby specialized species of higher trophic levels frequently show steeper species-area relationships and are particularly affected by habitat isolation (Kruess & Tscharntke 1994; Tscharntke et al. 2002a; Holt 2002; Steffan-Dewenter 2003). Further evidence for the trophic-level hypothesis is provided by the theory of island biogeography (Didham et al. 1996; Tscharntke & Kruess 1999).

Parasitoids are important in ecosystems as they can regulate population densities of their hosts. However, the importance of parasitoids for host population dynamics is unclear (Hunter 2001; Shaw et al. 2009). Land-use change, anthropogenic disturbance, habitat loss, and climate change are factors that might lead to (i) shifts in host-parasitoid interactions (Didham et al.1996; Steffan-Dewenter & Tscharntke 1999; Hance et al. 2007), (ii) alterations in host-parasitoid food webs (Tylianakis et al. 2007) or (iii) disruption of trophic interactions (Vanbergen et al. 2006).

The few studies conducted in this context focus on the effects of habitat loss and fragmentation on either herbivores or mutualists and their plant hosts, whereas data for host-antagonist interactions (herbivore, parasitoid/predator) are rare (Kruess & Tscharntke 1994; Zabel & Tscharntke 1998; Golden & Christ 1999; Komonen et al. 2000; Tscharntke et al. 2002b; Steffan-Dewenter 2003; van Nouhuys 2005; Dupont & Nielsen 2006; Valladares et al. 2006).
This study focuses on population densities and multitrophic interactions of the monophagous butterfly species *Polyommatus coridon* Poda which is restricted to

semi-natural grasslands with its larval host plant *Hippocrepis comosa* L., and its natural antagonists.
The following hypotheses are addressed in this study:

1. Population densities of all trophic levels increase with habitat area and habitat connectivity.
2. Higher trophic levels, particularly parasitoids of *P. coridon*, are more strongly affected by reduced habitat area and connectivity than lower trophic levels.

Material and Methods

Study region and study sites

The study region “Fränkische Schweiz” is located in the vicinity of the town of Bayreuth in northern Bavaria (Germany). Seventeen semi-natural grasslands along a habitat area and connectivity gradient were selected as study sites (Table 1). Semi-natural grasslands on shell limestone with numerous rocks are characteristic for the region (Böhmer 1994). The study sites were chosen to cover the full gradient in habitat area and connectivity in the study region, but being as similar as possible in habitat quality.

Study species

The Chalkhill Blue *Polyommatus coridon* Poda 1761 is a lycaenid butterfly which feeds as larvae exclusively on the Horseshoe Vetch *Hippocrepis comosa* L. 1753. Both species are specialized on calcareous grasslands (Asher et al. 2001; Van Swaay 2002). *P. coridon* is a univoltine species with high population densities (Weidemann 1995), and is distributed throughout southern and central Europe (Kudrna 2002). It is considered as a sedentary to moderately dispersing species (Settele et al. 2000; Asher et al. 2001; Cowley et al. 2001) and as a good indicator of habitat quality (Brereton et al. 2008). Most parasitoids of lycaenid butterflies belong to three insect families: Tachinidae (Diptera), Ichneumonidae (Hymenoptera), and Braconidae (Hymenoptera). Tachinidae and Braconidae parasitize larval stages and hatch out of the larva, whereas most Ichneumonidae parasitize larval stages and hatch out of the pupae. For *P. coridon* larvae the parasitoids *Cotesia saltatoria*, *Aleiodes bicolor* (Braconidae) (Baumgarten & Fiedler 1998), *Aplomya confinis* (Tachinidae) (Belshaw 1993; Baumgarten &

Fiedler 1998), *Ichneumon exilicornis* (Ichneumonidae) (Hinz & Horstmann 2007), and *Agrypon anomelas* (Ichneumonidae) (Horstmann et al. 1997) were recorded. Other possible parasitoid species of *P. coridon* are *Cotesia astrarches, Cotesia tenebrosa* and *Cotesia arctica* (Braconidae), and *Polytribax rufipes* (Ichneumonidae) (see www.taxapad.com).

The population size of the larval food plant *H. comosa* was estimated per study site when it was flowering in May 2008. The walked transects covered the total calcareous grassland sites and the size of each patch of *H. comosa* was recorded in m². In few study sites, where *H. comosa* was evenly distributed within the study sites a cover was estimated and also presented as m² within the study site.

Population density of adult *P. coridon* was estimated with transect walks, counting *P. coridon* individuals during the peak of the flight season (August 5th to 7th) 2008. Transect walks were divided into 5 min sub-transects, thereby the number of sub-transects varied between 4 and 18 sub-transects depending on the size of the habitat patch. Transect length ranged from 75 to 1800 m and the time spent on a site ranged from 20 to 90 min. Previous results showed that estimates of population densities are reasonably stable when surveys last at least 20 min (e.g. Kraus et al. 2004, 2005, Brückmann et al. 2010. Population densities of adult *P. coridon* were calculated by dividing the total number of individuals by the transect area (transect length*transect width). Adult *P. coridon* are evenly distributed throughout the calcareous grassland and not restricted to patches of its larval food plant *H. comosa.* The butterflies mainly search for flowering plants within the borders of the calcareous grasslands and use the same plants as roosting sites (personal observation). Therefore the density was calculated per site and not for the cover of *H. comosa* within the study sites. Additionally we counted the time needed for sampling the larvae per site and used the amount of larvae detected within one hour as a second estimation of butterfly population density.

P. coridon larvae were collected during daylight with sunny weather conditions from the beginning of May to the beginning of June 2008 when larvae were in the second to fourth larval stage. Larval detectability was similar for all study sites, except for a few sites where several days were needed to collect the minimum

number of larvae required. Reasons for this are e.g. food plants that are matted in the grass and not exposed on rocks, which made it more difficult to find the larvae underneath the plants. Larvae feed during the dawn and therefore can be found in the upper soil layer hiding from the sun (Ebert & Rennwald 1991).

Larvae were taken to the laboratory to measure body length on the sample day and to rear them separately in Petri-dishes at 23° C in climate chambers with 16 hours light per day and 70% humidity. Larvae were fed every second day until pupation with fresh leaves of *H. comosa* collected on the study sites. All collected larvae were controlled daily and reared until emergence of an adult butterfly or a parasitoid or until death in the Petri-dishes.

Habitat area, quality and connectivity

Habitat area, defined as patch size of the calcareous grassland, was estimated using orthorectified aerial photos of the year 2005. Habitat area ranged from 0.14 ha to 16.40 ha (mean 2.98 ± 1.04, Table 1). Habitat area and distances to adjacent calcareous grasslands were calculated with ArcView GIS 3.2 (ESRI 1995).

Study sites were chosen to be of similar habitat quality. All study sites were managed by grazing apart from one site which was only mown. None of the study sites was in an advanced succession stage (Table 1). Nearly all study sites were exposed to the south (Table 1).

Habitat connectivity (*CI*) for each of the 17 study sites (*i*) was measured edge-to-edge from the focal study site to the surrounding calcareous grasslands within a radius of 2 km using the equation of Hanski's Connectivity Index (Hanski 1994).

$$CI_i = \sum_{i \neq j} \exp(-\alpha\, d_{ij})\, A_j^b$$

A_j is the size (in m^2) of neighboring calcareous grasslands and d_{ij} is the distance (in km) from neighboring calcareous grasslands *j* to the study site *i*. The parameter α is a measure of the dispersal ability (1/average migration distance in km) and *b* is a parameter which scales the size of the surrounding habitat patches. We chose

α = 0.5, as we expect for *P. coridon* an average migration distance of 2 km, which is a good approximation for average dispersal of habitat specialized butterfly species of calcareous grasslands (Brückmann et al. 2010). Nonetheless assuming other dispersal abilities between 0.5 and 3 km does not change the connectivity index fundamentally (Krauss et al. 2003). For the scaling parameter *b* we chose *b* = 0.5 according to the assumptions of Moilanen and Nieminen (2002), who suggested that the ratio of patch edge to patch area decreases with $A^{0.5}$ when patch area increases. The same connectivity index was also used for *H. comosa* and the parasitoids, even though less information on dispersal ability was available. The connectivity gradient of our 17 landscapes ranged from 72.6 to 1579.4.

Statistical analysis

Simple regressions and t-tests were performed using the software SPSS 15.0.1 (SPSS Inc., 1989-2006), general linear models and logistic regressions for presence and absence of the two most abundant parasitoid species were calculated with R 2.10.1 (R Development Core Team 2009). Transformation of data was not necessary for response or explanatory variables. Response variables met the assumptions of normality and homoscedasticity in the statistical models. As explanatory variables we used: (1) habitat area and (2) connectivity index for all response variables, (3) the estimated population density of adult *P. coridon* (m^2) and (4) population density of *P. coridon* achieved by larval densities for parasitism rates and (5) plant cover of *H. comosa* (m^2) for parasitism rates and *P. coridon* densities. As response variables we used: (1) % parasitized *P. coridon*-larvae, (2) plant cover of *H. comosa* (m^2), (3) estimated population density of adult *P. coridon* (m^2) and (4) population density of the *P. coridon* estimated by larval densities. Mean body length (size) for *P. coridon* larvae per study site was used as Co-variable for the % parasitism models.

Table 1. The seventeen study sites with habitat area (ha), Hanski's Connectivity Index, and exposition and management; S = south-facing, T = top of the hill, W = west, G = grazed, M = mown, Gi = irregularly grazed.

no.	site	habitat area (ha)	connectivity index	exposition	management
1	Walberla	16.4	498.7	S, W	G
2	Hirschbach	2.7	1402.7	S	Gi
3	Pommelsbrunn	2.2	72.6	S	G, M
4	Heldmannsberg	0.2	418.1	S	M
5	Haselbrunn	3.9	1579.4	S	G
6	Kainach	0.3	1193.9	S, T	G
7	Hainbronn	2.8	171.5	S	Gi
8	Oberailsfeld	3.5	685.9	S	Gi
9	Kleinziegenfeld	10.8	771.6	S	G
10	Spitzberg	0.1	679.5	T	G
11	Neuhaus	1.1	277.2	W, T	Gi
12	Grossenohe	1.2	784.5	S	G
13	Urspring	0.8	288.7	S	G
14	Lindach	0.1	613	S	G
15	Roschlaub	1.9	344.8	S, T	Gi, M
16	Friesen	1.2	408.5	S, T	Gi
17	Goetzendorf	1.2	882.1	S, T	G

Results

In total 557 larvae of *P. coridon* were collected on the 17 study sites in April and May 2008. The number of collected larvae per study site ranged from 20 to 45 individuals, depending on the availability and the patchiness of the food plant. Five different parasitoid species hatched out of 61 *P. coridon* larvae and pupae belonging to the families of Braconidae, Ichneumonidae and Tachinidae. The overall rate of parasitism was 10%. A total of 47 larvae were parasitized by the solitary species *Cotesia saltatoria* Balevski 1980 (Braconidae), seven by the gregarious species *Cotesia arctica* Thomson 1895 (Braconidae), one by the solitary species *Aleiodes bicolour* Spinola 1808 (Braconidae), three by the solitary

species *Ichneumon exilicornis* Wesmael 1857 (Ichneumonidae) and three by the solitary species *Aplomya confinis* Fallen 1820 (Tachinidae).

Population size of the host plant *H. comosa* ranged between 35-1000 m^2 and was not significantly explained by habitat area or connectivity (Table 2, Fig. 1a, b).

Table 2. Regression analyses for % parasitized larvae, *P. coridon* (m^2), and *H. comosa* (m^2) with the explanatory variables habitat area, connectivity index, *H. comosa* (m^2), and *P. coridon* (m^2) (* $p < 0.05$).

		Predictor variables			
Dependent variables		**habitat area**	**connectivity index**	***H. comosa* (m^2)**	***P. coridon* (m^2)**
% parasitized	$F_{1,16}$	0.782	2.371	0.367	0.162
	p	0.390	0.144	0.553	0.693
	r	-0.223	0.369	0.155	0.103
***P. coridon* (m^2)**	$F_{1,16}$	0.832	6.987	0.286	-
	p	0.376	0.018*	0.601	-
	r	0.229	0.564	0.137	-
***H. comosa* (m^2)**	$F_{1,16}$	2.046	0.084	-	-
	p	0.173	0.776	-	-
	r	0.346	-0.075	-	-

Population densities of adult *P. coridon* ranged from 0.04 to 0.32 individuals per m^2 for transect data and were not significantly affected by habitat area or *H. comosa* population size but increased with increasing habitat connectivity (Table 2, Fig. 1c, d). Larval densities per hour searching time ranged between 1.8-27.3 individuals and did not correlate with the population density of adult *P. coridon* ($t = 0.29$, $df = 15$, $p = 0.78$) or any of the other predictor variables (results not shown).

The percentage of parasitized larvae was not significantly explained by habitat area, habitat connectivity, or population size or densities of *H. comosa* (m^2) or *P. coridon* (m^2), respectively (Table 2, Fig. 1e, f). The percentage of parasitized larvae in general linear models (Type I Sums of Squares) was explained by the Co-variable “mean larval size of *P. coridon*” (which was measured at the day when the larvae were collected) ($F_{3,13} = 5.15$, $p = 0.04$) but this did not essentially

change the impact of habitat area ($F_{3,13}$ = 2.98, p = 0.11) and the connectivity index ($F_{3,13}$ = 0.36, p = 0.55) on % parasitized larvae.

Presence and absence analysis (logistic regressions) of the two most abundant parasitoids *C. saltatoria* and *C. arctica* were not significant for the predictor variables *H. comosa*, *P. coridon* density, habitat area and connectivity (p > 0.24).

The impact of the predictor variables was also tested on the density of *C. saltatoria*, but neither the connectivity index ($F_{1,10}$ = 0.93, r^2 = 0.09, p = 0.36), *H. comosa* (m^2) ($F_{1,10}$ = 1.339, r^2 = 0.12, p = 0.27), *P. coridon* (m^2) ($F_{1,10}$ = 0.34, r^2 = 0.03, p = 0.57), nor habitat area ($F_{1,10}$ = 0.53, r^2 = 0.05, p = 0.49) significantly explained the density of *C. saltatoria*.

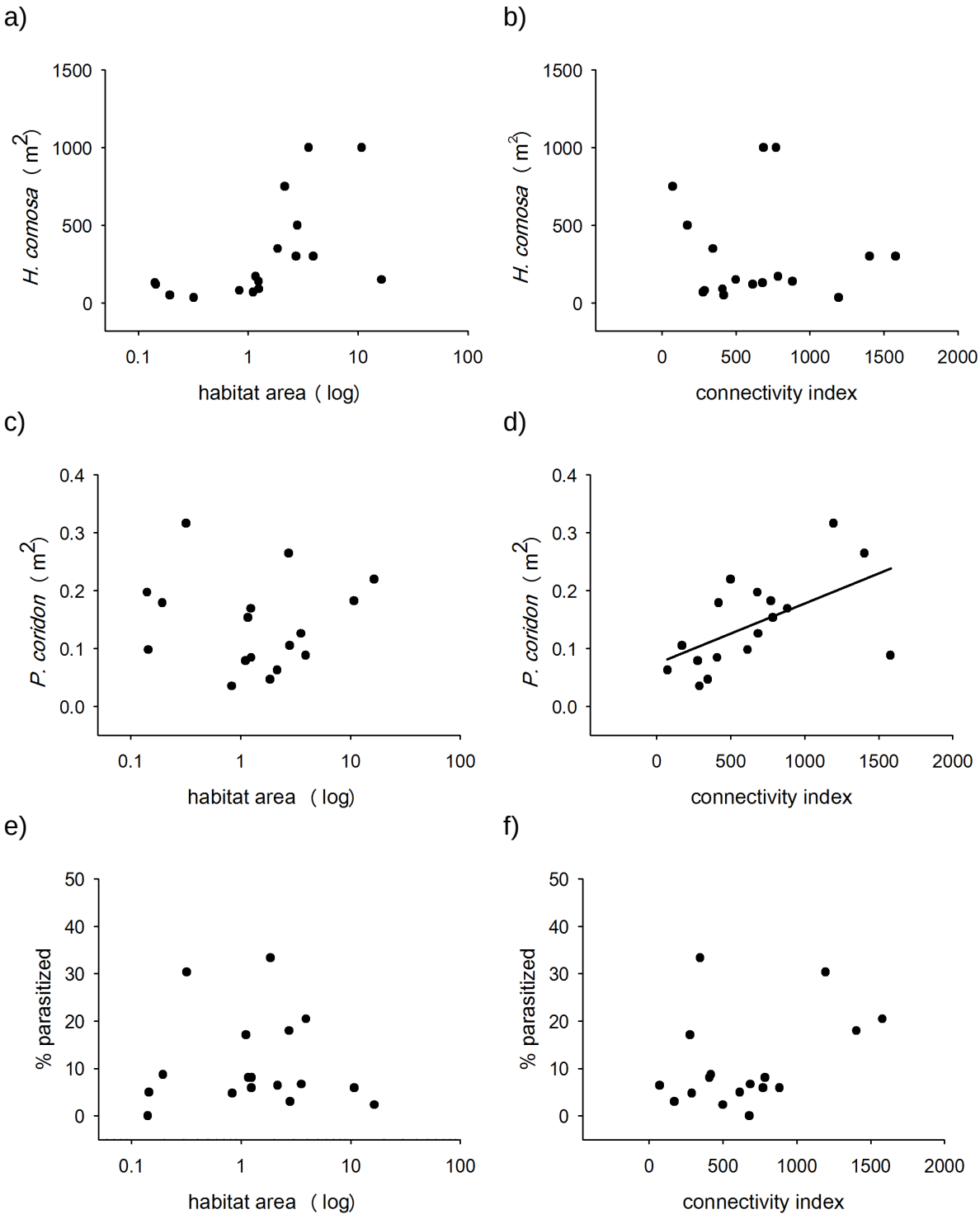

Fig. 1 Three trophic levels (plant, butterfly, parasitism rate) and their relationships with habitat area and connectivity (Statistics see Table 2).

a, b: No significant effect of habitat area and the connectivity index on the cover of the larval food plant *H. comosa*;

c, d: No significant effect of habitat area on the population density of *P. coridon*, but a significant effect of connectivity on population density of *P. coridon* ($y = 0.000104 x + 0.073$; $p = 0.018$);
e, f: No significant effect of habitat area and the connectivity index on % parasitized larvae of *P. coridon*.

Discussion

In this study we tested the response of three trophic levels, (1) the host plant *H. comosa*, (2) the specialized herbivore *P. coridon* and (3) its natural antagonists (parasitoids) to the loss of habitat area and connectivity in a region with highly fragmented calcareous grasslands. In contrast to our a priori hypothesis, % parasitism of *P. coridon* larvae did not depend on any of the tested explanatory variables. Percent parasitism decreased with increasing larval size which could be caused by extended collection time in some study sites. Larvae collected later could not have been parasitized by braconids or tachinids because these hatch out of early larval stages; thus these larvae could only have been parasitized by ichneumonids which hatch out of the pupae.

We further showed an impact of connectivity on the adult butterfly population density, suggesting that *P. coridon* is a sedentary species (Schmitt et al. 2006). For this reason increasing habitat loss and sustained fragmentation may impede between-patch migration leading to genetic impoverishment, inbreeding, loss of fitness and finally to population extinction (Saccheri et al. 1998; Schmitt et al. 2006). Unfortunately little is known about parasitoids of butterflies, except for well studied *Maculinea* species, species of the tribe Melitaeini and a few pest species e.g. of the genus *Pieris* (Shaw et al. 2009). Thereby information on the impact of non-biotic factors on the distribution of parasitoid species is lacking. In comparison to this study where habitat area had no effect on % parasitism, Doak (2000) found that a geometrid moth had the highest parasitism rates when habitat patches were small, and Cronin (2003) found that the abundance of egg parasitoids of the planthopper *Prokelisia crocea* increased with increasing habitat area but decreased with increasing patch isolation. We conclude that previous studies as well as our study are contradictory and do not generally confirm the prediction that

higher trophic levels are more sensitive to habitat loss and isolation than lower trophic levels.

Population density of *P. coridon* did not depend on the amount of the larval foodplant *H. comosa* in this study, but *P. coridon* certainly depends on the presence of the larval foodplant. In other studies, the amount of the larval foodplant explained the population density of *P. coridon* better. In contrast, habitat isolation had only little impact on the population density of *P. coridon* and no isolation-by-distance was found for the genetic differentiation of *P. coridon* (Krauss et al. 2004, 2005). It is generally hypothesized that the presence of the larval foodplant *H. comosa* is the limiting factor for re-colonization by *P. coridon* (Krauss et al. 2005; Brereton et al. 2008). But in our study mainly habitat connectivity played an important role for the population density of this specialized butterfly species. In contrast to the study by Krauss et al. (2005) where *P. coridon* was dependent on large habitats with large food plant populations, we found no effect of habitat area within our habitat area gradient. We therefore assume that the chosen habitat patches had a sufficient patch size to promote viable populations. Apart from habitat area and connectivity, habitat quality is a further important parameter for the persistence of butterfly populations in a fragmented landscape (Dennis & Eales 1999, Kuussaari et al. 2007). All study sites were selected to be similar in habitat quality (e.g. flower cover, management, exposition). Almost all study sites were grazed and south-facing (Table 1). However quality differences might be still caused by minor differences in management regimes (e.g. irregular management). Nonetheless habitat quality changes caused by different management were not found to have an effect on butterfly species richness in another study which included the sites from this study (Brückmann et al. 2010), similarly to a Swiss study where butterfly diversity did not differ between early fallows and managed pastures (Balmer & Erhardt 2000). Microclimatic conditions however can always play a role for the occurrence and population sizes of all three trophic levels tested in this study and we cannot rule out any possible bias due to different microclimates within and between the grasslands.

The parasitoids recorded on *P. coridon* larvae were endoparasites that are not specialized on *P. coridon* solely, but feed on other Lycaenidae species also.

Aleiodes bicolor (Braconidae) is known to parasitize several Lycaenidae in the *Polyommatus* group (e.g. *P. eros*, *P. icarus*, *P. artaxerxes*) (Baumgarten & Fiedler 1998). *Aplomya confinis* (Tachinidae) and *Cotesia saltatoria* (Braconidae) also feed on several species of the family Lycaenidae, e.g. *P. icarus*, *P. agestis*, *P. artaxerxes* (Baumgarten & Fiedler 1998; Menéndez et al. 2008), and *Ichneumon exilicornis* (Ichneumonidae) feeds on e.g. *P. argus*, *P. admetus* (Hinz & Horstmann 2007; Shaw et al. 2009). This leads to the assumption that many parasitoids might be able to use related hosts or even unrelated hosts in similar environments (Shaw et al. 2009) and are less restricted to the highly fragmented calcareous grasslands. In this case parasitoids could switch to different butterfly species belonging to their host range if a certain species is not present at a time. In our region at least *P. icarus* is also a very abundant butterfly species, with 2 – 3 generation per year (Brückmann et al. 2010). The lack of parasitoids specialized on *P. coridon* and the possibility of switching between different Lycaenidae-hosts makes it even more challenging to study the effects of habitat fragmentation on host-antagonist interaction webs of *P. coridon*. One would expect that parasitism based on parasitoids that are specialized for only one host-species might be strongly affected by increasing isolation of the focal habitat type, than parasitism based on parasitoids with a multi-host-system, and therefore not restricted to one habitat type (Anton et al. 2007). Since parasitoids of *P. coridon* are associated with other Lycaenidae that are not restricted to calcareous grasslands, extinction of parasitoid species might be unlikely even when habitat isolation increases. Alternatively, it might be that more specialized parasitoids of *P. coridon* already went extinct due to habitat fragmentation in the past. In general it is difficult to disentangle whether % parasitism per site depends on host density and seasonal changes, microsite selection of the host, or the impact of missing habitat connectivity or habitat area.

We found higher *P. coridon* densities of larvae on somewhat exposed host plants, either growing at edges of stones, brims or on parts that were slightly elevated and not infiltrated by grass and other plants as described by Ebert & Rennwald (1991). Therefore, such exposed host plant patches could serve as an attractant for parasitoids, in addition to volatiles or the presence of attendant ants (Pierce et al. 1987; Fiedler et al. 1992). This may indicate that the spatial structure of the habitat

also influences host-parasitoid interactions, where parasitoids in search of hosts can be affected by the distribution of the host, by the spatial arrangement of the host's microhabitats and by the spatial scale at which the parasitoid perceives variation in host abundance (Roland & Taylor 1997).

In conclusion, our data do not support the theory that species of higher trophic levels, at least parasitoids, are more affected by fragmentation than their host species. An obvious reason is that all recorded parasitoids in our study had a broader host range, while the butterfly host *P. coridon* was highly specialized and restricted to a single larval food plant. We found no impact of habitat area on the abundances of *H. comosa*, *P. coridon* or the parasitism rates. Only habitat connectivity was important for the population density of the studied specialized butterfly species, but not for the host plant or parasitoids. For the conservation of *P. coridon* we suggest a large-scale management of calcareous grasslands that are highly connected with adequate conditions for *H. comosa* to promote persistence of this threatened butterfly species. In contrast, parasitoids, despite their higher trophic level, need less conservation and management efforts, as long as they are able to use additional hosts from common butterfly species. For example, in our study sites and their surroundings, parasitoids can find alternative host species like the Common Blue, *Polyommatus icarus*. However, as environmental threats are increasing for all species and as biotic interactions between all species are unknown, our aim must be to protect valuable habitats like calcareous grasslands and their multi-trophic interactions.

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7. Impact of habitat fragmentation on population genetics

Fragmentation genetics of the grassland butterfly *Polyommatus coridon*: Stable genetic diversity or extinction debt?

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Abstract

Habitat fragmentation can have severe effects on the intraspecific variability of populations and thus plays a pivotal role in species conservation. Especially taxa with specific habitat demands and low dispersal behaviour suffer from habitat fragmentation. One such taxon, the Chalk-hill Blue butterfly, *Polyommatus coridon*, nowadays mostly occurs in small and isolated, calcareous grasslands across Central Europe. Here we investigate the population genetic structure of 15 local populations of this butterfly species over major parts of the Fränkische Schweiz (south-east Germany). Based on seven polymorphic microsatellites we estimate genetic diversity and differentiation. With use the data to test for potential effects of different habitat sizes, habitat connectivity, and population density. We found high genetic diversity but no significant genetic differentiation among the 15 local populations (F_{ST} = 0.0087, P > 0.05). Genetic diversity was not correlated with habitat size, habitat connectivity, or census population size. But, we found a marginally positive correlation between increasing habitat connectivity and population density (r^2 = 0.31, P < 0.05). Compared to data from other butterfly species, our data resemble a generalist species with well connected populations rather than a specialist taxon existing in a highly fragmented landscape. The high genetic diversity and the lack of differentiation might either be the result of relatively large and stable local populations and ongoing gene flow, or is the genetic legacy of formerly large and interconnected populations during periods of extensive agriculture.

Introduction

The fragmentation of habitats is known to have severe impacts on biodiversity. A common effect of fragmentation is a reduction of habitat size or even a complete loss of habitat patches, accompanied by an increase of geographic isolation of the remaining habitat patches (Fahrig et al. 2003). These effects have been analysed on the community level (e.g. Augenstein et al. 2012), species level (reviewed in Fahrig et al. 2003), as well as on the intraspecific level (e.g. Saccheri et al. 1998; Petit et al. 2003). Previous studies have shown that species with specific habitat needs (e.g. demanding a specific larval host plant) mostly suffer more severely under ongoing habitat fragmentation than generalists (Steffan-Dewenter & Tscharntke 2000; Anton et al. 2007; Brückmann et al. 2010a). The effects are

even more pronounced if the respective species have low dispersal abilities. Hence, the combination of specific habitat requirements and sedentary behaviour reduces landscape permeability and in turn increases the effects of fragmentation.

Molecular analyses have revealed that habitat fragmentation may lead to the subdivision of formerly interconnected populations as reflected by an increase in genetic differentiation resulting from reduced gene flow. A parallel decline in genetic diversity due to enhanced effects of genetic drift is often observed, especially in small and isolated populations (Keyghobadi et al. 2005). The loss of genetic variability in turn can lower the individual fitness and reduce the adaptability on environmental changes (Madsen et al. 2004). Thus, small and isolated populations have a higher risk of extinction due to stochastic events in comparisons to larger, well connected populations that are part of a meta-population network (Melbourne & Hastings 2008; Hunt & Bonsall 2009). Specialized species mostly exist in isolated populations, exhibit limited gene flow and have small effective population sizes. This situation can lead to higher genetic differentiation and a reduction of genetic diversity (Crnokrak & Barrett 2002; Louy et al. 2007; reviewed in Habel et al. 2013). In contrast, generalists have rather unspecific habitat demands, in consequence higher abundances and generally higher rates of gene flow between subpopulations. Thus, on the genetic level, generalists are often characterised by higher genetic diversity and lower genetic differentiation among local populations compared to habitat specialists (Louy et al. 2007; Dennis et al. 2011; Habel et al. 2013).

This knowledge can directly be translated into conservation management: Specialists can be conserved by keeping a high habitat quality in distinct conservation areas in which the species persist without being negatively affected from stochastic processes. In contrast, generalists still exist in high abundances, even in intensively used agricultural landscapes, maintaining a high level of genetic diversity by continuous gene flow (Habel & Schmitt 2012). In consequence, the perhaps most endangered group of species are ecologically “intermediate” species ranging between specialists and generalists. These species have specific habitat demands, but are not as restricted as real specialists and are genetically relatively diverse, almost like generalists. Species of this intermediate

group may suffer strongest under anthropogenic fragmentation as they are genetically not adapted to persist in highly isolated populations (Habel et al. 2013).

The Chalk-hill Blue, *Polyommatus coridon,* is one such intermediate species. This butterfly can be found in extensively used, calcareous grasslands, which today are highly restricted and often protected as nature reserves (Krauss et al. 2005; Brückmann et al. 2010a; Rosin et al. 2012). In this study, we analysed seven polymorphic microsatellites for 456 individuals of *P. coridon* collected at 15 locations scattered across the Fränkische Schweiz (south-east Germany). Based on our genetic and ecological data, we test for potential genetic effects of fragmentation on the intraspecific level and analyse genetic differentiation and genetic diversity. In detail we test if potential genetic effects are related with (i) habitat size, (ii) habitat isolation, and (iii) census population size of *P. coridon*.

Material and methods

Study species

The Chalk-hill Blue *Polyommatus coridon* is a univoltine butterfly mostly found at calcareous grasslands (Asher et al. 2001). It is classified as one of the most characteristic butterfly of this ecosystem (Van Swaay et al. 2006) and was thus frequently used as an indicator species for dry and hot calcareous grasslands in Central Europe (Brereton et al. 2008). The species´ distribution range mainly covers parts of Central Europe (Kudrna et al. 2011). The butterfly can exist in high population densities at some locations (up to 10.000 individuals / ha, Bink 1992; Thomas Schmitt, pers. comm.). Mark-Release-Recapture studies indicate that *P. coridon* is generally fairly sedentary (Settele et al. 2000; Asher et al. 2001; Cowley et al. 2001) with few individuals migrating longer distances (Ebert & Rennwald 1991) up to 20km (Asher et al. 2001). Recent destruction of extensively used calcareous grasslands has had negative effects on the abundance of the butterfly, which led to its addition to several red lists of threatened species (e.g. Reinhardt et al. 2009).

Study region and sampling

The study region “Fränkische Schweiz” is located in south-eastern Germany, covering a total geographic extent of 2600 km^2 (Fig. 1).

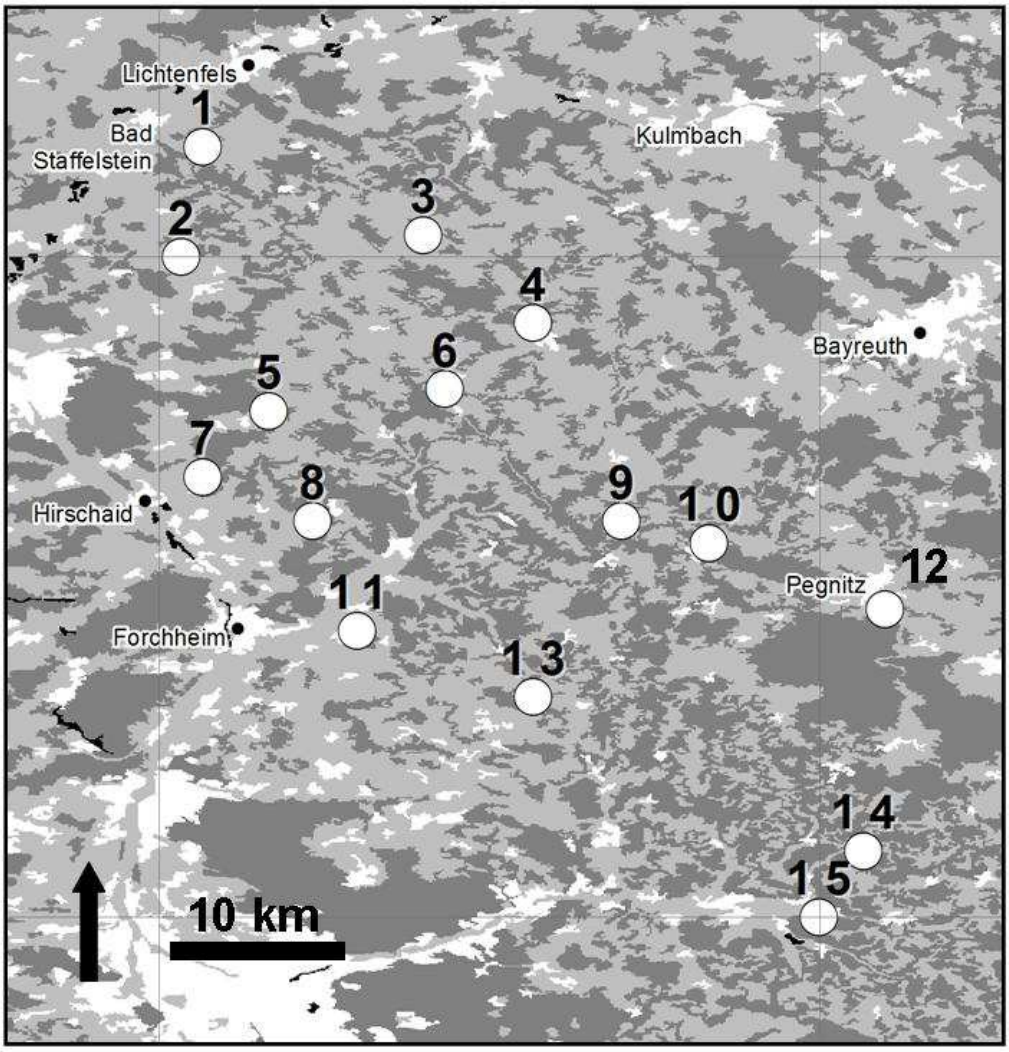

Fig. 1 Location of the study region Fränkische Schweiz in Germany (A) and the 15 sampling sites (B): Spitzberg (1), Roschlaub (2), Kleinziegenfeld (3), Kainach (4), Lindach (5), Neuhaus (6), Friesen (7), Goetzendorf (8), Oberailsfeld (9), Haselbrunn (10), Walberla (11), Hainbronn (12), Großenohe (13), Hirschbach (14), Pommelsbrunn (15). Numbers of localities coincide with other figures and tables.

About 95% of the formerly existing calcareous grasslands of our study region were destroyed since the middle of the 19th century due to forestation, fertilization, the abandonment of historic land use practices and subsequent succession of open vegetation (Böhmer 1994). In consequence, the few remaining extensively used grassland patches represent small and isolated habitats, mostly protected as

nature reserves (Krebs et al. 1999; Benton et al. 2003; Krauss et al. 2010; Stork & Waits 2010). These calcareous grasslands belong to the most species-rich habitats for plants and invertebrates in Europe (Wallis De Vries 2002). We selected 15 study sites with populations of *P. coridon* being characterised by different habitat size, different degrees of habitat connectivity, and varying population densities (Fig. 1, Table 1).

Table 1 Overview of all 15 sampling sites with habitat and population characteristics. Given are locality with a running number (coinciding with other tables and figures), GPS coordinates (N, E), habitat size (ha), connectivity index (CI) (*sensu* Hanski et al. 1994), population density (per m^2), and the number of individuals sampled and analysed.

Locality – Site Nr.	Nr.	GPS (N/E)-coordinates	habitat size [ha]	CI	Individuals / m^2	N
Spitzberg – SPI	1	50°5'; 11°2'	0.1	679.5	0.20	29
Roschlaub – ROS	2	50°0'; 11°1'	1.9	344.8	0.05	31
Kleinziegenfeld - KLE	3	50°1'; 11°12	10.8	771.6	0.18	32
Kainach – KAI	4	49°57'; 11°17'	0.3	1193.9	0.32	32
Lindach – LIN	5	49°53'; 11°5'	0.1	613	0.10	30
Neuhaus – NEU	6	49°54'; 11°13'	1.1	277.2	0.08	26
Friesen – FRI	7	49°50'; 11°2'	1.2	408.5	0.08	31
Goetzendorf – GOE	8	49°48'; 11°7'	1.2	882.1	0.17	30
Oberailsfeld – OBE	9	49°48'; 11°21'	3.5	685.9	0.13	32
Haselbrunn – HAS	10	49°47'; 11°25'	3.9	1579.4	0.09	28
Walberla – WAL	11	49°43'; 11°9'	16.4	498.7	0.22	29
Hainbronn –HAI	12	49°44'; 11°33'	2.8	171.5	0.11	29
Großenohe – GRO	13	49°40'; 11°17'	1.2	784.5	0.15	29
Hirschbach – HIR	14	49°33'; 11°32'	2.7	1402.7	0.26	30
Pommelsbrunn – POM	15	49°30'; 11°30'	2.2	72.6	0.06	29

A total of 456 individuals of *P. coridon* were collected from these 15 locations. Sampling sizes varied from 26 to 32 individuals per site. Only males were sampled to prevent negative effects on the population persistence. Individuals were netted in August 2008 during the peak of the flight period. Samples were immediately frozen in liquid nitrogen and stored at -80°C until molecular analyses.

Designing of microsatellite primers

Species specific primers for microsatellites were developed by Ecogenics GmbH (Zürich, Switzerland). An enriched library was constructed from size selected genomic DNA, ligated to SNX forward / SNX reverse-linkers (Hamilton et al. 1999), and enriched by magnetic bead selection with biotin-labelled $(CT)_{13}$, $(GT)_{13}$, $(GTAT)_7$, and $(GATA)_7$ oligonucleotide repeats (Gautschi et al. 2000a,b). Out of 528 recombinant colonies screened, we received a positive signal for 340 clones after hybridization. Plasmids from 156 positive clones were sequenced and primers were designed for 27 microsatellite inserts, of which 15 were tested for polymorphisms. Four monomorphic microsatellites and three microsatellites highly affected by genotyping errors were excluded from further analyses. Subsequently we used the 8 remaining microsatellites for molecular analyses (see below).

Molecular analyses

DNA was isolated from legs of *P. coridon* using the NucleoMag 96 Tissue DNA isolation kit (Macherey-Nagel, Düren, Germany). DNA isolation was conducted according the manufacturer's protocol. Microsatellite loci were amplified and fluorescently labelled by PCR using the procedure described by Schuelke (2000). In this procedure the forward primers contained an 18-bp M13 tail serving as target for the labelled M13 primer. The PCR reaction contained 0.04 µM forward primer, 0.16 µM reverse primer, 0.16 µM M13 primer labelled with BMN5, BMN6, or DY751, respectively (Biomers, Ulm, Germany), 1 µl Multiplex PCR Master mix and 1 µl Q-Solution (Multiplex PCR Kit, Qiagen, Hilden, Germany). Finally, we added 1 µl genomic DNA of *P. coridon*. PCR products were run on a CEQ8000 capillary electrophoresis system (Beckman-Coulter, Krefeld, Germany) to identify the lengths of microsatellite fragments. Further details on PCR protocols like primer-specific annealing temperatures and multiplexing, as well as information on fragment length detection are given in Supplementary Material Appendix S1.

Population genetic analyses

Only loci that could be scored unambiguously were used in the analyses. Deviations from Hardy-Weinberg-equilibrium (HWE) and linkage disequilibrium were assessed using GENEPOP 4.1 (Raymond & Rousset 1995; Rousset 2008). We used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to detect potential

effects from large allele dropout and the presence of null alleles. Null allele frequencies were calculated based on the individual inbreeding model (IIM) implemented in the software INEST 2.0 (Inbreeding / Null Allele Estimation) (Chybicki & Burczyk 2009). MICROCHECKER identified significant excess of heterozygosity due to the presence of null alleles, with a frequency of null alleles per locus ranging from 0.02 to 0.37 and a mean frequency over all loci ranging from 0.11 to 0.17 depending on the population (null allele frequency per locus and per population are given in Supplementary Material Appendix S2). Especially high frequencies of null alleles were found in locus Polcor21 (0.37), Polcor23 (0.23), and Polcor26 (0.12). Thus, Polycor21 was excluded from further analysis. For all remaining loci, significant null allele frequencies were adjusted using MICRO-CHECKER. Subsequent analyses were performed using this adjusted microsatellite data matrix consisting of 7 microsatellites.

Allele frequencies, the mean number of alleles (*A*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated with ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010). Allelic richness was calculated based on the smallest sample size (here 26 individuals) using FSTAT 3.1 (Goudet 1995). Non-hierarchical analyses of molecular variance AMOVA and pairwise F_{ST} values were calculated with ARLEQUIN. Inbreeding coefficients using the original (uncorrected) and the null allele corrected (i.e. F_{IS} and $F_{IS;IIM}$) data set were calculated with INEST. The correction of inbreeding coefficients and calculation of corrected allele frequencies were conducted based on the IIM with 10,000 iterations and a burn-in of 1000 steps. To test for a correlation between genetic and geographic distances (isolation-by-distance), we used pairwise F_{ST} values and conducted a Mantel-test with the program ZT (Van de Peer 2002).

Habitat and population characteristics

The 15 selected study sites differed in size, geographic isolation, connectivity and population density. Habitat size was estimated with ARCVIEW GIS 3.2 (ESRI 1995) using orthorectified digital aerial photographs taken in the year 2005. Habitat connectivity of each study site was calculated using Hanski's Connectivity Index (Hanski 1994):

$$CI_i = \sum_{i \neq j} \exp(-\alpha\, d_{ij})\, A_j^b$$

This index considers the presence of all calcareous grasslands within a radius of 2km around the centre of the focal site, the dispersal ability, habitat area and the total distance among patches (Hanski 1994). A_j is the size (in m^2) of neighbouring calcareous grasslands, d_{ij} the distance (in km) from neighbouring calcareous grasslands *j* to the study site *i*. The parameter α is a measure of the dispersal ability (1/average migration distance in km), and *b* is a parameter which scales the size of the surrounding habitat patches. We expect an average migration distance for *P. coridon* of 2km per population per year. This value represents the mean migration distance of several butterfly species specialized to calcareous grasslands (Brückmann et al. 2010a, b). Accordingly *α* was chosen as 0.5. Nonetheless, assuming other dispersal distances, e.g. between 0.5km and 3km suggested from community studies, does not change the connectivity index fundamentally (Krauss et al. 2003). We chose *b* = 0.5 according to the assumptions of Moilanen and Nieminen (2002) suggesting that the ratio between habitat edge to habitat size decreases with A = 0.5 when patch size increases.

Population density of *P. coridon* was estimated by transect walks, counting individuals of the butterfly during the peak of its flight season (August 5^{th} to 7^{th}) during the year 2008. Depending on the habitat size, transect walks were divided into 5 min sub-transects, with a total of 4-18 sub-transects per site. Transect length ranged from 75 to 1800 m and the time spent on a site ranged from 20 to 90 min depending on transect length i.e. habitat size. The transect width was 5 m on each side. Previous results showed that estimates of population densities are reasonably stable when surveys last at least 20 min (e.g. Krauss et al. 2004, 2005; Brückmann et al. 2010). Population densities of adult *P. coridon* were then calculated as the total number of individuals divided by the transect area (transect length*transect width). To approximate actual population sizes, the population densities were multiplied by the factor 6.1. This factor is derived from a previous mark-release-recapture study, which revealed that ‘real’ population densities of *P. coridon* are 6.1 times higher than population sizes based on transect walks (Krauss et al. 2004). The population densities were further used to estimate the census population size (N_c) by simple extrapolation across the habitat area.

Comparative analyses of ecological and genetic data

Transformation of the data was not necessary as our model residuals met the assumptions of normality and homoscedasticity. Linear and multiple regressions and Pearson correlations were performed to test for potential effects from habitat size, habitat connectivity, and individual density on all genetic parameters calculated (population specific *A*, *AR*, *He*, *Ho*, F_{ST}, F_{IS}, *Nc*). Analyses were performed using the software SPSS 15.0.1 (SPSS Inc., 1989-2006).

Results

Genetic diversity and genetic differentiation

The mean number of alleles per locus and site ranged from 11 to 25. Of the 105 tests of conformity to Hardy-Weinberg equilibrium (HWE), 32 deviated significantly from HWE after Bonferroni correction due to heterozygosity deficiency. However, no locus or population showed systematic deviations. None of the seven loci showed significant linkage disequilibrium after Bonferroni correction.

Genetic diversity was relatively similar across all 15 populations, with a mean number of alleles *A* = 19.22 (±1.11), allelic richness *AR* = 17.75 (±0.91), expected heterozygosity *He* = 91.0% (±0.67) and observed heterozygosity *Ho* = 74.3% (±2.72). Population specific values showed no significant deviations for any parameters of genetic diversity among all local populations (Kruskal-Wallis-ANOVA, $P > 0.05$). Loci and population specific information on diversities and allele frequencies are given in Table 2 and Supplementary Material Appendix S3 and S4.

Table 2 Parameters of genetic diversity and census population size (N_c) of each analysed *Polyommatus coridon* population. The following parameters are given: mean number of alleles (*A*), allelic richness (calculated based on the lowest number of individuals sampled for a population, here 21 individuals) (*AR*), observed heterozygosity (H_o), expected heterozygosity (H_e), and the genetic variance found within individuals (here the Wright's inbreeding coefficient F_{IS}, F = 1 - *H*o / *H*e, and $F_{IS;IMM}$, corrected for null alleles based on the individual inbreeding model IIM, Cybicki and Burczyk 2009).

Locality	**A**	**AR**	H_o **[%]**	H_e **[%]**	F_{IS}	$F_{IS;IIM}$	**SE** $_{FIS;IMM}$	N_c
Spitzberg -1	20.00	18.74	76.3	91.9	0.18	0.017	0.018	200
Roschlaub - 2	19.86	18.18	71.6	91.1	0.21	0.028	0.028	950
Kleinziegenfeld – 3	20.29	17.97	72.9	91.0	0.19	0.021	0.023	19440
Kainach - 4	17.14	15.66	72.2	89.9	0.17	0.015	0.017	960
Lindach – 5	19.86	18.28	75.7	91.1	0.16	0.012	0.014	100
Neuhaus – 6	18.29	17.77	76.3	91.7	0.17	0.021	0.022	880
Friesen – 7	20.86	18.66	78.4	91.4	0.14	0.013	0.014	960
Goetzendorf – 8	18.00	16.52	74.3	89.9	0.17	0.017	0.019	2040
Oberailsfeld – 9	20.14	18.38	69.7	91.6	0.18	0.033	0.030	4550
Haselbrunn – 10	20.29	18.69	78.2	91.7	0.15	0.019	0.020	3510
Walberla – 11	18.57	17.22	71.9	90.4	0.18	0.027	0.025	36080
Hainbronn – 12	19.29	18.25	73.4	90.8	0.19	0.017	0.020	3080
Großenohe – 13	18.71	17.46	75.5	91.1	0.16	0.020	0.021	1800
Hirschbach – 14	19.43	17.98	70.8	91.8	0.20	0.017	0.019	7020
Pommelsbrunn - 15	17.57	16.45	76.9	90.2	0.15	0.011	0.013	1320
Mean (±SD)	19.22 (±1.11)	17.75 (±0.91)	74.27 (±2.72)	91.04 (±0.67)	0.17 (±0.02)	0.02 (±0.01)	0.020 (±0.00)	-

The overall genetic differentiation (AMOVA) was not significant, with a genetic variance of 0.0229 (F_{ST} = 0.0087, P > 0.05) found among populations, a genetic variance of 0.3287 (F_{IS} = 0.1261, P < 0.0001) detected among individuals within populations, and a genetic variance of 2.2774 restricted to single individuals. Pairwise F_{ST} values ranged from 0.0000 to 0.0293. The Mantel-test revealed a lack of isolation-by-distance (Mantel-test: P = 0.223). Inbreeding coefficients ranged from 0.1422 to 0.2198. Inbreeding coefficients corrected for potential null alleles and large allele dropout were lower ranging from 0.0113 to 0.0335 as null alleles and genotyping errors may cause a decline in the level of heterozygosity and thus impact inbreeding coefficients (cf. Van Oosterhout et al. 2004). Null alleles are not uncommon in butterflies because of high variation in the flanking regions of microsatellite repeats (reviewed in Meglecz et al. 2004). The presence of null alleles may further affect F-statistics (Chapuis and Estoup 2007), but the bias that they introduce is considered to be negligible when frequencies are low (<0.2) (Dakin & Avise 2004).

Genetic population structure and spatial habitat data

A correlation matrix of the independent habitat factors, habitat size, habitat isolation i.e. connectivity index, and population density (individuals per m^2) showed no significant relationship with any parameter of genetic diversity and molecular variance (inbreeding coefficient). The only significant correlation indicated that local population density increased with better habitat connectivity (P = 0.03, r^2 = 0.31). Multiple regression analysis did not yield any significant results either, indicating no relationship of the two habitat predictors (habitat size, connectivity index) and the genetic variables (H_e, $F_{IS;\,IIM}$) (p > 0.1).

Discussion

Ecological specialist with high genetic diversity

The genetic diversity found for *P. coridon* was high in comparison with other butterfly species such as the specialist species *Lycaena helle* (Finger et al. 2009), *Parnassius smintheus* (Keyghobadi et al. 2002), or the closely related sibling *Polyommatus bellargus* (Harper et al. 2003), that were all analysed using the same marker system (Table 3). The study on *P. bellargus* was conducted in the UK, the northernmost distribution margin of this butterfly potentially indicating

stronger effects from more pronounced demographic and environmental stochasticity at the margin of the distribution range (for general reviews see Hampe & Petit 2005; Melbourne & Hastings 2008). Moreover, the British populations of *P. bellargus* were subjected to a population bottleneck in the 1970s. Both factors may have resulted in the comparatively lower genetic diversity found for *P. bellargus* at its distribution edge (Harper et al. 2003). Other genetic studies of *P. coridon* using allozymes and covering a larger geographic area show similar high levels of genetic diversity (Schmitt & Seitz 2002a; Krauss et al. 2004). The genetic diversity in this species exceeds that of other Lycaenid butterflies that are also characterised by specific habitat demands, such as representatives of the genus *Maculinea* (Gadeberg & Boomsma 1997; Bereczki et al. 2005; Pecsenye et al. 2007a, b), *Aricia agestis*, *Cupido minimus* (Habel & Schmitt 2009), or the highly endangered *Lycaena helle* (Habel et al. 2011) (an overview is given in Table 3). The relatively high genetic diversity found in *P. coridon*, however, does not match with the current habitat distribution being characterized by small habitat remnants and high geographic isolation. Previous studies analysing the generalist–specialist continuum indicated that high genetic diversity is usually found in generalist species characterized by panmictic distributions and high abundances. High interconnectivity leads to continuous gene flow which allows species to maintain a high levels of genetic diversity (Habel & Schmitt 2012; Habel et al. 2013, with references therein, see also the introduction section). In contrast, specialists are assumed to exist in distinct populations and thus are more strongly affected by genetic drift effects and the loss of genetic information (Crnokrak & Barrett 2002; Frankham et al. 2002).

Table 3 Ecological specialization and genetic diversity based on A) allozymes and B) microsatellites of various lycaenid butterflies. Given are four ecological classifications following Bink (1992) (dispersal behaviour, food preference (i.e. larval food plant specialisation, egg-laying behaviour, potential number of eggs); the original classifications were transformed into a 0-1 matrix; the lower the value, the more specialized is the butterfly species. The general classification is the mean value calculated from the four ecological characters. Three parameters of genetic diversity (numbers of alleles *A*, expected heterozygosity *He* (or observed heterozygosity *), and percentage of polymorphic loci *P* including the respective reference). Abbreviations: A: Austria, B: Belgium, CE: Central Europe, CZ: Czech Republic, D: Germany, DK: Denmark, EU: Europe, F: France, H: Hungary, P: Poland, L: Luxembourg, RO: Romania, FC: Fennoscandia.

Species	**Area**	**Mean of 4 parameters**	**Food preference**	**Egg laying**	**dispersal**	**Egg number**	**A**	***He* [%]**	***P* [%]**	**Reference**
A) Allozymes										
Cupido minimus	D, F, L	0.086	0	0	0.2	0.143	2.24±0.22	18.3±1.6	73.5±16.4	Habel and Schmitt 2009
Maculinea nausithous	H	0.142	0	0	0	0.571	1.50±0.095	10.1±1.4*	31.0±5.84	Pecsenye et al. 2007a
Maculinea teleius	H	0.193	0	0	0.2	0.571	1.71±0.041	8.9±0.6*	42.3±2.53	Pecsenye et al. 2007a
Maculinea alcon	DK	0.194	0	0.148	0.2	0.428	1.48±0.12	15.4±21.4	32.2	Gadeberg & Boomsma 1997
Maculinea alcon alcon	H	0.194	0	0.148	0.2	0.428	1.38±0.069	8.4±1.1*	28.6±4.50	Pecsenye et al. 2007a
Maculinea alcon rebeli	H	0.194	0	0.148	0.2	0.428	1.47±0.073	8.0±1.0*	32.7±4.28	Pecsenye et al. 2007a
Lycaena helle	F, B	0.278	0	0.571	0.4	0.142	1.46±0.12	5.8±2.4	-	Habel et al. 2010b
Lycaena tityrus	D, A	0.349	0	0.571	0.4	0.428	1.90	14.6	50.0	Karl et al. 2009
Maculinea arion	H	0.404	0.5	0.148	0.4	0.571	1.70±0.095	19.4±1.4 *	54.8±5.84	Pecsenye et al. 2007a

Polyommatus bellargus	EU	0.439	0.5	0.429	0.4	0.429	2.70±0.44	20.5±2.1	81.3±4.8	Schmitt & Seitz 2001
Polyommatus coridon	CE	0.525	0.5	0.429	0.6	0.571	2.68±0.33	20.0±1.6	74.2±9.2	Schmitt & Seitz 2001
Polyommatus icarus	CE	0.525	0.5	0.429	0.6	0.571	2.97±0.26	17.7±1.1	77.9±8.1	Schmitt et al. 2003
Aricia agestis	D, F, L	0.614	1	0.286	0.6	0.571	2.12±0.10	15.7±1.4	52.1±4.2	Habel and Schmitt 2009
Aricia artaxerxes	H	0.614	1	0.286	0.6	0.571	2.66	24.6	57.3	Pecsenye et al. 2007b
B) Microsatellites										
Maculinea teleius	FC	0.142	0	0	0	0.571	5.00±4.20	46.5±33.1; 44.4±33.4*	-	Zeisset et al. 2005
Maculinea alcon	FC	0.194	0	0.148	0.2	0.428	6.50±5.44	45.8±43.0; 38.2±38.7*	-	Zeisset et al. 2005
Maculinea arion	P	0.194	0	0.148	0.2	0.428	15.80	74.9; 62.4*	-	Rutkowski et al. 2009
Maculinea rebeli	P	0.194	0	0.148	0.2	0.428	3.80	38.0; 13.0*	-	Rutkowski et al. 2009
Maculinea arion	P	0.194	0	0.148	0.2	0.428	21.40	76.6; 69.1*	-	Sielezniew & Rutkowski 2012
Maculinea arion	FC	0.194	0	0.148	0.2	0.428		52.3±10.9*	-	Ugelvig et al. 2012
Lycaena helle	EU, FC	0.278	0	0.571	0.4	0.142	8.20±0.90	68.0±3.0; 62.0±4.0*	-	Habel et al. 2011
Polyommatus bellargus	UK	0.439	0.5	0.429	0.4	0.429		68.7±4.8*	-	Harper et al. 2003
Polyommatus coridon (own data)	D	0.525	0.5	0.429	0.6	0.571	19.22±1.11	91.0±0.7; 74.3±2.7*	-	Habel et al. 2014 (this contribution)

Our data for *P. coridon* hence provide a mixed message: On the one hand, the species has relatively strict habitat demands and a patchy habitat distribution, which would suggest a status as a specialist. On the other hand, the genetic data show high diversity and low differentiation such as usually found in generalists. Three non-mutually exclusive hypotheses can explain this pattern: (i) The current genetic diversity is the result of relatively large population sizes which prevent genetic drift effects (supported by high N_c estimates of this study); (ii) few individuals are still migrating among patches preventing differentiation and maintaining high levels of genetic diversity; or (iii) the current genetic diversity is a relict of past times when *P. coridon* populations occurred in well connected grassland habitats in the region. The third scenario would imply that a major proportion of current genetic diversity represents an extinction debt that will be paid in the future.

However, the lack of genetic differentiation may also be explained by the high variability of the genetic markers used in the study. Hence, the lack of differentiation could either represent a biological phenomenon or may be an artefact of the hypervariable microsatellite markers, raising the question if the selected marker is a good choice to test for genetic differentiation over restricted spatial scales. In the future less variable markers such as single nucleotide polymorphisms (SNPs) may provide a better alternative. However, despite the developments of genotyping by sequencing for non-model organisms (Davey et al. 2011; Narum et al. 2013) these markers remain relatively difficult to obtain and expensive. Until SNPs become fully accessible for all researchers, microsatellites represent a good alternative if analysed correctly (Keller et al. 2004; Selkoe & Toonen 2006). As we analysed the data with caution and adjusted for genotyping errors and null alleles we consider the results as valid and not artefacts.

Gene flow and large population sizes

Polyommatus coridon genetically represents a situation of population interconnectivity despite the patchy distribution of its few remaining habitats and relatively low dispersal rates. A Mark-Release-Recapture campaign showed that the major proportion of a local population of *P. coridon* remains within the same habitat patch without migrating to adjacent habitats (Schmitt et al. 2006). In

accordance, this butterfly has been classified as a sedentary species (Bink 1992). However, Schmitt and colleagues also indicated that a small number of individuals are migrating to neighbouring habitat patches (Schmitt et al. 2006). Theoretical models have indicated that even such low numbers of migrants can effectively prevent populations from diverging (Slatkin 1987). In addition, our transect data indicated moderate to high population densities. This interpretation suggests that population sizes are large enough and gene flow between populations is sufficient to maintain high levels of genetic diversity without geographical differentiation.

We found no significant relationship of any genetic parameter to habitat characteristics, geographic distance, or to population density. A similar lack of isolation-by-distance has been shown for *P. coridon* even on greater spatial scales (2.3-147.2 km, Schmitt & Seitz 2002a). Other studies, however, detected isolation-by-distance on a regional scale at the margin of the distribution range (Krauss et al. 2004) and on a continental scale (here within the Eastern European lineage, Schmitt & Seitz 2002b). In contrast, studies on other specialist butterfly species showed significant isolation-by-distance patterns, even on a landscape level; these species include *P. bellargus* (Harper et al. 2003), *Parnassius smintheu*s (Keyghobadi et al. 2002) and *Lycaena helle* (Finger et al. 2009). The lack of isolation-by-distance provides further evidence that (i) habitat isolation does not prevent gene flow between locations, and/or that (ii) populations are large and thus genetic drift plays a negligible role (see also Schmitt & Seitz 2002a); further, this result may indicate that (iii) habitat isolation in *P. coridon* is very recent and time has not been sufficient to lead to genetic differentiation (see below).

Extinction debt of genetic diversity

Compared with other lycaenid butterflies, the genetic diversity detected for *P. coridon* is very high, for both, allozymes and microsatellites (Table 3a, b); yet the specific habitat demands and the current distribution of *P. coridon* suggest that this butterfly species is a habitat specialist. The observed pattern might arise from a time lag. The genetic information still represents the formerly widespread distribution of *P. coridon* when it occurred in most extensively used pastures that in the meanwhile vanished. A meta-analysis on 22 population genetic datasets of lepidopterans based on polymorphic allozymes over similar landscape scales in Central Europe revealed a similar picture for various butterfly species (Habel et al.

2013). Species like *Zygaena loti*, *Thymelicus acteon* and *Cupido minimus* still had high levels of genetic diversity, yet existed in highly fragmented environments (Habel et al. 2013, with references therein). This situation might be a critical signal as such “intermediate” species are not adapted to live in geographic isolation with small population sizes and might even suffer stronger under ongoing fragmentation processes than specialist species.

While species extinction debt is in the meanwhile widely known and acknowledged to be one of the major challenges in biodiversity conservation (Kuussaari et al. 2009), our example of *P. coridon* suggests that, on a molecular level, many species might show an extinction debt of genetic diversity. Thus, from a population genetic point of view, conservation actions should take the species-specific population structure and the species´ history into account. Whether the current local population sizes and their dispersal capacity are sufficient to counteract genetic drift and the loss of genetic diversity in the future remains an open question. Therefore we recommend appropriate local and landscape scale management to maintain habitat quality, improve habitat connectivity and preserve the intraspecific variability.

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Supplementary Material Appendix S1 Characteristics of eleven polymorphic microsatellite loci in *Polyommatus coridon.* Given is name of locus, GenBank accession number, primer sequence (Abbreviations: F – forward primer; R – reverse), Repeat motif, size of sequenced allele in base pairs (bp), annealing temperature (T_a), PCR-multiplex, colours used, and the used capillary for electrophoresis.

Locus	Primer sequences (5'-3)'	Repeat motif	Size of sequenced allele (bp)	T_a (°C)	PCR-Multiplex	Color	Sequencing
Polcor 02	F: ATGCAGATGCAGATGTGGAG R: GATGAACGAAAACCTCAATGG	$(AC)_{16}(AGAC)_3$	136-180	56	-	BMN-6	CE-Pc-1
Polcor 03	F: GTCGCCAGGATCAACAGTG R: TCGGGCTTGTAGTTCCAGAG	$(TATG)_{15}$	121-319	60	-	DY751	CE-Pc-1
Polcor 05	F: GACACGCACACATGCAAATAG R: GCTTTGGAAAAGTTCGGATG	$(AC)_{22}$	126-164	56	1	BMN-6	CE-Pc-2
Polcor 11	F: CGTAGGTAGACACGTCTGTGTATATG R: CGCCGCTATACAACGTGAG	$(GT)_5(CT)_{15}(GT)_{14}$	87-169	60	2	BMN-5	CE-Pc-1
Polcor 12	F: CTATCTGCTCGCGGATGAAG R: GAATCCGTGGAAGTGTCGTC	$(AC)_6GC(AC)_4GA(CA)_{15}$	184-238	60	2	BMN-5	CE-Pc-1
Polcor 13	F: TGAAAGATCGCGACTAAATTG R: CCCTCACAAACAATGGACTAAC	$(CA)_4TA(CA)_{14}$	135-197	56	-	BMN-5	CE-Pc-2
Polcor 18	F: GCGTTTGGCACGTTTATCTC R: ATCATGGGACGACGCATAC	$(GT)_6(GCGT)_2(GT)4GAAT(GT)_8$	149-185	50	-	DY751	CE-Pc-2

Polcor 21	F: TTCAGGACTCCAGGTTCCAC R: CTCAAACGTTGGCTGCAAG	$(TG)_{14}$	111-187	56	-	BMN-5	CE-Pc-3
Polcor 23	F: GGTGATCGCGACAACAATAG R: ACGCCGTTCTGGAAGACAC	$(AC)_{20}$	179-267	56	1	BMN-6	CE-Pc-2
Polcor 24	F: CCGAAGATGAAGCAAACTACAAG R: TTTCCTGCTGGAATACATGC	$(TACA)_{9}$	155-197	50	-	BMN-6	CE-Pc-3
Polcor 26	F: TCATAACCGATGCCACAGTC R: TTACCTTTCGGTTGGAGGTC	$(CA)_{19}$	165-289	56	-	DY751	CE-Pc-3

Supplementary Material Appendix S2 Null allele frequency per locus and per population calculated based on the Expectation Maximization (EM) algorithm according to Dempster et al. (1977), using the programme FreeNa (Chapuis and Estoup 2007). Numbers of populations coincide with figures and tables in the manuscript.

Population	PC12	PC02	PC03	PC13	PC05	PC23	PC21	PC26
1	0.03	0.00	0.04	0.15	0.06	0.07	0.29	0.22
2	0.00	0.06	0.11	0.12	0.11	0.06	0.43	0.31
3	0.03	0.06	0.03	0.12	0.03	0.12	0.32	0.23
4	0.00	0.00	0.06	0.13	0.04	0.15	0.57	0.26
5	0.00	0.00	0.05	0.22	0.06	0.06	0.40	0.20
6	0.00	0.03	0.04	0.18	0.06	0.10	0.34	0.12
7	0.01	0.00	0.04	0.11	0.07	0.09	0.33	0.23
8	0.00	0.06	0.05	0.12	0.01	0.14	0.41	0.16
9	0.05	0.06	0.12	0.09	0.10	0.29	0.32	0.31
10	0.02	0.00	0.03	0.11	0.04	0.14	0.33	0.17
11	0.00	0.10	0.07	0.08	0.03	0.17	0.41	0.29
12	0.07	0.00	0.13	0.14	0.12	0.16	0.33	0.30
13	0.02	0.05	0.00	0.12	0.08	0.10	0.33	0.24
14	0.14	0.06	0.08	0.16	0.00	0.11	0.33	0.19
15	0.00	0.12	0.08	0.09	0.00	0.05	0.34	0.26
mean	0.02	0.04	0.06	0.13	0.05	0.12	0.37	0.23

Supplementary Material S3 Population- and locus-wise genetic diversity including all populations and individuals analysed.

Locus	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean	SD
PC02	A	16	16	14	16	16	17	16	16	17	19	17	18	14	16	16	16.27	1.24
	AR	11.48	11.02	12.16	7.63	8.52	9.77	11.53	8.59	9.85	10.92	9.22	8.53	14.07	9.16	10.38	10,19	1,70
	Ho	0.90	0.81	0.75	0.97	0.93	0.85	0.97	0.80	0.81	0.89	0.72	0.93	0.76	0.90	0.72	0.85	0.08
	He	0.92	0.91	0.91	0.94	0.93	0.93	0.92	0.91	0.93	0.94	0.94	0.94	0.90	0.92	0.94	0.93	0.01
	F_{IS}	0.03	0.12	0.17	-0.03	-0.01	0.09	-0.05	0.12	0.13	0.05	0.23	0.01	0.16	0.02	0.23	0.08	0.09
PC03	A	25	27	25	23	30	25	22	23	29	21	19	24	25	27	26	24.73	2.82
	AR	14.92	14.72	12.98	15.52	15.27	16.67	14.59	15.33	16.02	18.497	16.36	17.18	13.27	15.094	15.52	15,46	1,40
	Ho	0.86	0.74	0.84	0.77	0.87	0.85	0.87	0.83	0.77	0.89	0.79	0.92	0.93	0.77	0.79	0.83	0.06
	He	0.96	0.97	0.94	0.93	0.97	0.95	0.93	0.95	0.96	0.95	0.93	0.96	0.94	0.96	0.96	0.95	0.01
	F_{IS}	0.10	0.23	0.11	0.17	0.11	0.11	0.07	0.13	0.19	0.06	0.14	0.04	0.01	0.20	0.17	0.12	0.06
PC05	A	18	17	21	16	20	19	23	14	20	16	20	18	19	18	18	18.47	2.16
	AR	22.62	24.24	21.84	19.73	26.90	24.05	19.88	21.16	25.47	19.901	17.59	23.52	22.29	23.87	24.19	22,48	2,50
	Ho	0.74	0.71	0.81	0.84	0.77	0.73	0.87	0.80	0.72	0.82	0.90	0.69	0.76	0.90	0.93	0.80	0.07
	He	0.90	0.91	0.90	0.91	0.90	0.88	0.92	0.84	0.93	0.91	0.92	0.93	0.89	0.93	0.89	0.90	0.02
	F_{IS}	0.18	0.22	0.10	0.08	0.15	0.18	0.10	0.05	0.23	0.09	0.02	0.26	0.15	0.03	-0.05	0.12	0.08
PC12	A	12	12	14	8	9	10	13	9	11	11	10	9	16	10	11	11.00	2.07
	AR	19.08	18.31	20.42	18.88	17.77	18.45	19.07	16.47	18.74	18.21	21.96	19.48	17.82	20.51	13.14	18,55	1,99
	Ho	0.79	0.84	0.75	0.72	0.87	0.81	0.74	0.80	0.84	0.79	0.86	0.71	0.86	0.50	0.79	0.78	0.09
	He	0.81	0.81	0.81	0.76	0.77	0.84	0.79	0.81	0.76	0.79	0.76	0.69	0.86	0.80	0.79	0.79	0.04
	F_{IS}	0.02	-0.03	0.07	0.06	-0.12	0.04	0.06	0.01	-0.12	0.01	-0.14	-0.02	0.00	0.37	0.00	0.01	0.12
PC13	A	20	21	23	21	19	19	21	18	21	20	24	20	19	22	14	20.13	2.25
	AR	17.27	15.83	18.18	14.59	17.84	18.43	20.14	12.56	17.93	14.87	17.89	16.86	16.28	16.79	16.31	16,78	1,84

	Ho	0.79	0.65	0.68	0.67	0.50	0.58	0.80	0.70	0.73	0.71	0.79	0.66	0.75	0.63	0.69	0.69	0.08
	He	0.94	0.89	0.94	0.93	0.94	0.94	0.94	0.91	0.93	0.92	0.96	0.94	0.92	0.95	0.89	0.93	0.02
	F_{IS}	0.17	0.28	0.27	0.29	0.47	0.38	0.15	0.23	0.21	0.22	0.17	0.30	0.19	0.33	0.23	0.26	0.08
PC23	A	20	21	17	16	19	17	22	19	18	18	18	18	19	19	16	18.47	1.63
	AR	19.05	18.71	15.70	14.84	16.75	16.76	20.09	17.59	17.39	17.23	16.56	17.30	17.83	17.81	15.64	17,28	1,36
	Ho	0.78	0.81	0.77	0.61	0.73	0.84	0.74	0.63	0.56	0.67	0.59	0.70	0.72	0.69	0.79	0.71	0.08
	He	0.93	0.91	0.92	0.89	0.89	0.92	0.94	0.92	0.94	0.93	0.91	0.94	0.94	0.92	0.90	0.92	0.02
	F_{IS}	0.17	0.11	0.16	0.32	0.18	0.10	0.20	0.31	0.41	0.28	0.36	0.25	0.23	0.25	0.12	0.23	0.09
PC26	A	29	25	28	20	26	21	29	27	25	32	22	26	21	24	20	25.00	3.54
	AR	26.52	24.41	24.52	18.40	24.90	20.29	25.34	23.96	23.24	31.19	20.92	24.88	20.68	22.61	20.00	23,46	3,18
	Ho	0.52	0.46	0.50	0.47	0.63	0.69	0.50	0.63	0.45	0.63	0.48	0.48	0.60	0.57	0.62	0.55	0.08
	He	0.97	0.97	0.96	0.93	0.97	0.95	0.96	0.95	0.96	0.98	0.94	0.97	0.95	0.96	0.94	0.96	0.01
	F_{IS}	0.57	0.52	0.48	0.29	0.35	0.27	0.48	0.34	0.24	0.36	0.49	0.50	0.37	0.23	0.33	0.39	0.11
	A (mean)	20.00	19.86	20.29	17.14	19.86	18.29	20.86	18.00	20.14	19.57	18.57	19.00	19.00	19.43	17.29	-	-
	F_{IS} (mean)	0.18	0.21	0.19	0.17	0.16	0.17	0.14	0.17	0.18	0.15	0.18	0.19	0.16	0.20	0.15	-	-
	Ho (mean)	0.76	0.72	0.73	0.72	0.76	0.76	0.78	0.74	0.69	0.77	0.73	0.73	0.77	0.71	0.76	-	-
	He (mean)	0.92	0.91	0.91	0.90	0.91	0.92	0.92	0.90	0.92	0.92	0.91	0.91	0.91	0.92	0.90	-	-
	$F_{IS;\ IIM}$	0.02	0.03	0.02	0.02	0.01	0.02	0.01	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.01	-	-
	N	29	31	32	32	30	26	31	30	32	28	29	29	29	30	29	29.8	1.60

Supplementary Material S4 Allele frequencies calculated for all populations analysed and for each single locus separately. Given is Locus, Allele, and the size of each allele (fragment length).

Locus	Allele	Size	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Polycor 12	1	203	0.0345	0.0323	0.0312	0.0000	0.0000	0.0769	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0536	0.0000	0.0000
	2	205	0.0000	0.0000	0.0156	0.0000	0.0167	0.0000	0.0161	0.0000	0.0000	0.0000	0.0161	0.0179	0.0000	0.0167	0.0667
	3	207	0.0517	0.0806	0.0156	0.0781	0.1333	0.0769	0.0484	0.0833	0.0484	0.0167	0.0484	0.0536	0.0714	0.0833	0.0667
	4	209	0.0345	0.0161	0.0469	0.0312	0.0500	0.0962	0.1290	0.0833	0.0484	0.0667	0.0645	0.0357	0.1250	0.1000	0.0500
	5	211	0.2241	0.3226	0.3125	0.1719	0.2500	0.2692	0.2419	0.3000	0.2742	0.2500	0.2581	0.5000	0.2143	0.3667	0.4167
	6	213	0.3621	0.2742	0.2969	0.4219	0.3833	0.2500	0.3710	0.2833	0.4032	0.3333	0.4194	0.2321	0.2857	0.2167	0.1667
	7	215	0.1207	0.0806	0.0938	0.1562	0.0833	0.1346	0.0484	0.0667	0.0806	0.1500	0.0968	0.0893	0.0357	0.1333	0.0500
	8	217	0.0000	0.0484	0.0156	0.0000	0.0000	0.0192	0.0000	0.1000	0.0161	0.0333	0.0000	0.0000	0.0357	0.0333	0.0167
	9	219	0.0172	0.0161	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000	0.0000
	10	221	0.0345	0.0000	0.0156	0.0000	0.0167	0.0000	0.0161	0.0000	0.0161	0.0000	0.0000	0.0357	0.0179	0.0000	0.0167
	11	223	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167
	12	225	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	13	227	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	14	229	0.0000	0.0161	0.0469	0.0312	0.0000	0.0192	0.0000	0.0167	0.0161	0.0167	0.0323	0.0179	0.0179	0.0167	0.0000
	15	241	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	16	243	0.0000	0.0161	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	17	245	0.0345	0.0484	0.0000	0.0000	0.0333	0.0192	0.0161	0.0167	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000
	18	247	0.0345	0.0484	0.0625	0.0000	0.0000	0.0000	0.0161	0.0000	0.0645	0.0167	0.0161	0.0000	0.0357	0.0167	0.0833
	19	249	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0333	0.0000	0.0000	0.0357	0.0000	0.0000
	20	251	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000

	21	253	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0000	0.0167	0.0161	0.0000	0.0179	0.0000	0.0000
	22	255	0.0345	0.0000	0.0156	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0500	0.0000	0.0000	0.0179	0.0000	0.0000
	23	257	0.0000	0.0000	0.0000	0.0938	0.0333	0.0385	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000	0.0500
	24	291	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000	0.0000	0.0000
	25	297	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Polycor 2	1	155	0.0000	0.0161	0.0000	0.0312	0.0500	0.0385	0.0000	0.0000	0.0000	0.0167	0.0172	0.0333	0.0000	0.0172	0.0345
	2	157	0.0000	0.0161	0.0156	0.0312	0.0000	0.0385	0.0161	0.0333	0.0312	0.0500	0.0345	0.0167	0.0000	0.0517	0.0000
	3	159	0.0667	0.0968	0.0000	0.0781	0.1000	0.0385	0.0161	0.0333	0.0781	0.0833	0.1034	0.0333	0.0500	0.1552	0.0690
	4	161	0.0167	0.0645	0.0156	0.0625	0.0333	0.0192	0.0968	0.0333	0.0469	0.0833	0.0345	0.0500	0.0333	0.0000	0.0172
	5	163	0.1333	0.0806	0.1250	0.0469	0.0667	0.1923	0.0806	0.0333	0.0469	0.0333	0.0862	0.0167	0.1833	0.0690	0.0690
	6	165	0.0167	0.0000	0.0000	0.0000	0.0667	0.0385	0.0323	0.0500	0.0000	0.0167	0.0172	0.0000	0.0000	0.0690	0.0172
	7	167	0.0833	0.0161	0.0781	0.0625	0.1167	0.0769	0.0968	0.1167	0.0625	0.0500	0.0517	0.1167	0.1167	0.1552	0.0690
	8	169	0.1167	0.0323	0.1094	0.0156	0.1500	0.0962	0.1290	0.2167	0.0938	0.0667	0.1379	0.0500	0.1000	0.0517	0.0862
	9	171	0.0667	0.1613	0.2031	0.0312	0.1333	0.1154	0.0806	0.1333	0.0938	0.1333	0.0690	0.0500	0.0500	0.0345	0.0690
	10	173	0.1500	0.1613	0.1094	0.0469	0.0167	0.0769	0.0968	0.1000	0.1406	0.1000	0.1034	0.1000	0.2000	0.1379	0.0862
	11	175	0.1000	0.0645	0.0625	0.1094	0.0667	0.0385	0.0806	0.0500	0.0625	0.0167	0.0345	0.0500	0.1000	0.0172	0.1034
	12	177	0.0833	0.0806	0.0781	0.0625	0.0167	0.0769	0.1613	0.0333	0.0625	0.0833	0.0862	0.1667	0.0333	0.0517	0.1207
	13	179	0.0333	0.0323	0.0625	0.0781	0.0333	0.0000	0.0161	0.0167	0.1250	0.0500	0.0517	0.0500	0.0333	0.0000	0.0345
	14	181	0.0500	0.1129	0.0625	0.1094	0.0500	0.0192	0.0484	0.0167	0.0312	0.0667	0.0690	0.0833	0.0167	0.0862	0.1207
	15	183	0.0167	0.0000	0.0156	0.0000	0.0333	0.0000	0.0161	0.0667	0.0156	0.0000	0.0000	0.0167	0.0000	0.0000	0.0345
	16	185	0.0000	0.0000	0.0000	0.0469	0.0500	0.0577	0.0161	0.0000	0.0312	0.0333	0.0000	0.0333	0.0000	0.0517	0.0000
	17	187	0.0333	0.0323	0.0469	0.1094	0.0000	0.0000	0.0000	0.0333	0.0469	0.0500	0.0345	0.0667	0.0500	0.0000	0.0345
	18	189	0.0000	0.0000	0.0156	0.0781	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0517	0.0000	0.0167	0.0172	0.0000
	19	191	0.0167	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0156	0.0000	0.0000	0.0333	0.0167	0.0000	0.0000

	20	193	0.0167	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0172	0.0000
	21	195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0345
	22	197	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0172	0.0000	0.0000	0.0000	0.0000
	23	199	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	24	201	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	25	203	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0172	0.0000
	26	205	0.0000	0.0000	0.0000	0.0000	0.0000	0.0385	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	27	207	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Polycor 3	1	140	0.0167	0.0000	0.0484	0.0161	0.0167	0.0000	0.0000	0.0000	0.0323	0.0000	0.1000	0.0000	0.0167	0.0167	0.0000
	2	144	0.0000	0.0161	0.0161	0.0161	0.0000	0.0192	0.0000	0.0000	0.0161	0.0000	0.0667	0.0000	0.0000	0.0000	0.0167
	3	148	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0179	0.0000	0.0000	0.0000	0.0000	0.0000
	4	152	0.0167	0.0323	0.0161	0.0000	0.0167	0.0577	0.0000	0.0333	0.0161	0.1071	0.0500	0.0741	0.0333	0.0667	0.0500
	5	156	0.1333	0.0484	0.1613	0.0484	0.0333	0.0192	0.0323	0.0500	0.1129	0.0714	0.1667	0.0370	0.1333	0.1167	0.0833
	6	160	0.0500	0.0806	0.0161	0.0161	0.0333	0.0385	0.0968	0.0333	0.0323	0.0536	0.0500	0.0926	0.0000	0.0667	0.0833
	7	164	0.0833	0.0484	0.0968	0.0161	0.0667	0.0769	0.0161	0.0667	0.0806	0.0179	0.1000	0.0185	0.1000	0.0500	0.0833
	8	168	0.0500	0.0000	0.0000	0.0806	0.0500	0.1731	0.0645	0.0333	0.0161	0.0357	0.0333	0.0926	0.0333	0.0667	0.0500
	9	172	0.0500	0.0161	0.1290	0.1452	0.0167	0.0385	0.0968	0.0667	0.0484	0.0893	0.0167	0.0741	0.0333	0.0833	0.0000
	10	176	0.0500	0.0968	0.0645	0.1613	0.0167	0.0000	0.1935	0.0833	0.0645	0.1429	0.1333	0.0556	0.0167	0.0333	0.0167
	11	180	0.0667	0.0484	0.0161	0.0806	0.0500	0.0000	0.0323	0.0167	0.0161	0.0179	0.0333	0.0370	0.1667	0.0000	0.0667
	12	184	0.0000	0.0484	0.0323	0.0000	0.0000	0.0192	0.0000	0.1167	0.0000	0.0536	0.0167	0.0000	0.0000	0.0000	0.0500
	13	188	0.0833	0.0645	0.0484	0.0161	0.0833	0.0192	0.0484	0.0000	0.0323	0.0714	0.0000	0.0370	0.0167	0.0833	0.1000
	14	192	0.0167	0.0161	0.0000	0.0000	0.0167	0.0577	0.0000	0.0333	0.0806	0.0179	0.0333	0.0000	0.0167	0.0333	0.0333
	15	196	0.0000	0.0484	0.0000	0.0000	0.0000	0.0000	0.0000	0.0500	0.0000	0.0357	0.0000	0.0000	0.0000	0.0667	0.0167
	16	200	0.0167	0.0484	0.0000	0.0161	0.0000	0.0192	0.0484	0.0000	0.0484	0.0179	0.0000	0.0185	0.0000	0.0167	0.0333

17	206	0.0500	0.0323	0.0161	0.0000	0.0667	0.0000	0.0161	0.0667	0.0161	0.0357	0.0000	0.0000	0.0333	0.0000	0.0333
18	210	0.0333	0.0000	0.0000	0.0161	0.0500	0.0192	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0167	0.0500
19	214	0.0333	0.0161	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0185	0.0000	0.0000	0.0167
20	218	0.0000	0.0323	0.0484	0.0323	0.0333	0.0000	0.0000	0.0167	0.0484	0.0357	0.0000	0.0185	0.0000	0.0000	0.0000
21	222	0.0000	0.0645	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0536	0.0167	0.0000	0.0333	0.0000	0.0167
22	226	0.0000	0.0000	0.0000	0.0161	0.0167	0.0385	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
23	230	0.0000	0.0484	0.0161	0.0806	0.0333	0.0000	0.0000	0.0000	0.0323	0.0179	0.0167	0.0000	0.0333	0.0000	0.0167
24	234	0.0333	0.0000	0.0645	0.0000	0.0167	0.0000	0.0323	0.0167	0.0323	0.0536	0.0167	0.0926	0.0000	0.0167	0.0000
25	238	0.0667	0.0323	0.0161	0.1129	0.0500	0.0769	0.0323	0.0000	0.0000	0.0000	0.0333	0.0556	0.0167	0.0500	0.0333
26	242	0.0000	0.0000	0.0323	0.0161	0.0500	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0556	0.0167	0.0000	0.0167
27	246	0.0167	0.0000	0.0323	0.0000	0.0667	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0333	0.0167
28	250	0.0167	0.0000	0.0000	0.0161	0.0167	0.0385	0.0161	0.0667	0.0645	0.0000	0.0000	0.0000	0.0167	0.0000	0.0167
29	254	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0161	0.0000	0.0000	0.0185	0.0000	0.0167	0.0000
30	258	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000
31	260	0.0000	0.0161	0.0323	0.0000	0.0000	0.0385	0.0323	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000
32	264	0.0000	0.0000	0.0161	0.0000	0.0333	0.0000	0.0000	0.0333	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
33	268	0.0167	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0185	0.0000	0.0167	0.0000
34	272	0.0000	0.0161	0.0000	0.0161	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
35	276	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000
36	280	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
37	284	0.0167	0.0161	0.0161	0.0000	0.0167	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000
38	288	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
39	292	0.0000	0.0484	0.0161	0.0323	0.0000	0.0192	0.0000	0.0000	0.0161	0.0000	0.0167	0.0000	0.0833	0.0000	0.0333
40	296	0.0167	0.0000	0.0161	0.0161	0.0000	0.0000	0.0161	0.0000	0.0161	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000
41	300	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0167	0.0000	0.0000	0.0667	0.0000	0.0167	0.0167	0.0000

	42	304	0.0167	0.0161	0.0161	0.0161	0.0167	0.0385	0.0000	0.0000	0.0161	0.0000	0.0000	0.0556	0.0000	0.0000	0.0000
	43	308	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0357	0.0000	0.0185	0.0000	0.0167	0.0167
	44	312	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.1000	0.0161	0.0000	0.0000	0.0185	0.0000	0.0167	0.0000
	45	316	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0167	0.0167
	46	318	0.0000	0.0000	0.0000	0.0000	0.0000	0.0385	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	47	320	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0161	0.0000	0.0000	0.0000	0.0000	0.0185	0.0500	0.0000	0.0000
	48	324	0.0000	0.0000	0.0000	0.0000	0.0000	0.0577	0.0645	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000
	49	326	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	50	328	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167
	51	330	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000	0.0000	0.0000	0.0000	0.0000
	52	334	0.0000	0.0161	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	53	338	0.0167	0.0000	0.0161	0.0000	0.0167	0.0192	0.0645	0.0167	0.0323	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000
	54	342	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0167
	55	346	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	56	348	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
	57	356	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000
Polycor 13	1	137	0.0000	0.0000	0.0312	0.0000	0.0167	0.0000	0.0167	0.0167	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000
	2	141	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000
	3	145	0.0000	0.0323	0.0156	0.0167	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0667	0.0000
	4	149	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5	155	0.0185	0.0484	0.0156	0.0667	0.0667	0.0000	0.0667	0.0000	0.0500	0.0333	0.0667	0.0000	0.0172	0.0500	0.0000
	6	157	0.0370	0.0161	0.0625	0.0167	0.0833	0.0192	0.0167	0.0667	0.0167	0.0000	0.0333	0.0500	0.0000	0.0167	0.2500
	7	159	0.0370	0.0968	0.0312	0.1333	0.0333	0.1731	0.0833	0.0500	0.1333	0.0667	0.1000	0.1833	0.1034	0.1000	0.0500
	8	161	0.0556	0.0806	0.0156	0.0000	0.0667	0.0577	0.0333	0.1500	0.0167	0.0167	0.0167	0.0833	0.2069	0.1167	0.0500

9	163	0.0556	0.0161	0.0469	0.0167	0.0333	0.0769	0.0500	0.0167	0.0500	0.0167	0.0167	0.0667	0.0517	0.0333	0.0000
10	165	0.0741	0.0806	0.0625	0.0167	0.0833	0.0769	0.1333	0.0333	0.1000	0.0333	0.0500	0.0500	0.0862	0.0167	0.1333
11	167	0.1481	0.2742	0.1719	0.1667	0.1333	0.0769	0.0833	0.2167	0.0500	0.1000	0.0833	0.0667	0.1034	0.0667	0.0000
12	169	0.1111	0.0161	0.0625	0.0167	0.1000	0.0577	0.1000	0.0167	0.0667	0.0833	0.0333	0.0667	0.0345	0.0333	0.1167
13	171	0.0926	0.1129	0.0625	0.0833	0.1167	0.0577	0.1167	0.0167	0.1667	0.2333	0.1000	0.0500	0.0345	0.1167	0.0500
14	173	0.0185	0.0161	0.0000	0.0167	0.0000	0.0385	0.0167	0.0000	0.0000	0.0000	0.0167	0.0000	0.0345	0.0000	0.0333
15	175	0.0741	0.0000	0.0000	0.0667	0.0333	0.0192	0.0333	0.0000	0.0333	0.0167	0.0167	0.0167	0.0000	0.0167	0.0167
16	177	0.0000	0.0161	0.0000	0.0333	0.0000	0.0192	0.0167	0.0500	0.0167	0.0833	0.0333	0.0333	0.0000	0.0000	0.0000
17	179	0.0185	0.0161	0.1094	0.0667	0.0167	0.0962	0.0667	0.0833	0.1167	0.0500	0.0667	0.0500	0.0690	0.0500	0.0000
18	181	0.0741	0.0323	0.0625	0.0833	0.0667	0.0769	0.0333	0.0167	0.0167	0.0833	0.0667	0.0167	0.0517	0.0333	0.0833
19	183	0.0000	0.0161	0.0312	0.0167	0.0167	0.0192	0.0000	0.0000	0.0000	0.0167	0.0667	0.0000	0.0000	0.0500	0.0000
20	185	0.0185	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0167	0.0000	0.0000	0.0000
21	187	0.0556	0.0000	0.0000	0.0167	0.0333	0.0000	0.0167	0.0333	0.0000	0.0333	0.0333	0.0167	0.0000	0.0167	0.0000
22	189	0.0185	0.0000	0.0156	0.0000	0.0000	0.0192	0.0167	0.0167	0.0167	0.0000	0.0000	0.0500	0.0000	0.0333	0.1167
23	191	0.0370	0.0323	0.0156	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0172	0.0500	0.0500
24	193	0.0000	0.0323	0.0625	0.0333	0.0000	0.0385	0.0000	0.1167	0.0500	0.0167	0.0500	0.0000	0.0345	0.0167	0.0167
25	195	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0500	0.0000	0.0167	0.0333	0.0000	0.0500	0.0172	0.0000	0.0167
26	197	0.0000	0.0000	0.0156	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0167	0.0167	0.0000	0.0517	0.0000	0.0167
27	199	0.0185	0.0161	0.0000	0.0000	0.0000	0.0192	0.0167	0.0000	0.0167	0.0000	0.0167	0.0500	0.0000	0.0000	0.0000
28	201	0.0185	0.0161	0.0000	0.0667	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0333	0.0000
29	203	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000
30	205	0.0000	0.0161	0.0000	0.0000	0.0333	0.0000	0.0167	0.0000	0.0167	0.0000	0.0333	0.0000	0.0000	0.0333	0.0000
31	207	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
32	209	0.0000	0.0000	0.0312	0.0000	0.0000	0.0000	0.0000	0.0167	0.0167	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000
33	211	0.0185	0.0161	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0167	0.0000	0.0333	0.0000

	34	213	0.0000	0.0000	0.0312	0.0000	0.0167	0.0000	0.0000	0.0000	0.0167	0.0000	0.0167	0.0000	0.0172	0.0167	0.0000
	35	215	0.0000	0.0000	0.0156	0.0000	0.0000	0.0385	0.0000	0.0500	0.0000	0.0167	0.0000	0.0333	0.0345	0.0000	0.0000
	36	221	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	37	223	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000
	38	225	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Polycor 5	1	138	0.0000	0.0161	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	2	144	0.0926	0.0645	0.0469	0.1250	0.0833	0.0192	0.0500	0.0667	0.0938	0.0833	0.0484	0.0833	0.0167	0.1000	0.1000
	3	146	0.2593	0.1935	0.2344	0.1719	0.1833	0.3077	0.1667	0.2000	0.1719	0.2000	0.1774	0.1667	0.3167	0.1667	0.0667
	4	148	0.1111	0.1290	0.1250	0.1406	0.2167	0.0769	0.1833	0.3167	0.0625	0.1833	0.1774	0.1667	0.0667	0.0833	0.1500
	5	150	0.0370	0.0161	0.0156	0.0000	0.0333	0.0385	0.0500	0.0000	0.1094	0.0333	0.0323	0.0000	0.0667	0.1167	0.0500
	6	152	0.0926	0.0484	0.0781	0.0625	0.0167	0.0000	0.0167	0.0000	0.0312	0.0833	0.0161	0.0333	0.0000	0.0333	0.0167
	7	154	0.0370	0.0323	0.0469	0.0625	0.0333	0.0385	0.0500	0.0167	0.0625	0.0667	0.0806	0.0667	0.1000	0.1333	0.0333
	8	156	0.0556	0.1613	0.1562	0.0312	0.0833	0.0577	0.1167	0.0500	0.1094	0.0500	0.0161	0.0667	0.0333	0.0500	0.2500
	9	158	0.0185	0.0323	0.0000	0.0469	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	10	160	0.0000	0.0484	0.0312	0.0312	0.0500	0.0385	0.0333	0.0167	0.0781	0.0333	0.0161	0.0000	0.0667	0.0333	0.0833
	11	162	0.0370	0.0000	0.0312	0.0938	0.0833	0.0962	0.0000	0.0000	0.0469	0.1000	0.0323	0.0500	0.0167	0.0000	0.0500
	12	164	0.0000	0.0161	0.0156	0.0000	0.0167	0.0000	0.0000	0.0000	0.0156	0.0000	0.0323	0.0667	0.0333	0.0500	0.0500
	13	166	0.0000	0.0323	0.0312	0.0000	0.0167	0.0192	0.0167	0.1167	0.0000	0.0000	0.0161	0.0167	0.0000	0.0167	0.0167
	14	168	0.0556	0.0484	0.0156	0.0469	0.0167	0.0192	0.0167	0.0000	0.0000	0.0000	0.0323	0.0500	0.0000	0.0167	0.0333
	15	170	0.0185	0.0000	0.0156	0.0156	0.0167	0.0385	0.0667	0.0000	0.0156	0.0500	0.1129	0.0333	0.0167	0.0167	0.0000
	16	172	0.0370	0.0000	0.0156	0.0156	0.0167	0.0385	0.0167	0.0000	0.0000	0.0333	0.0000	0.0000	0.0167	0.0333	0.0000
	17	174	0.0000	0.0000	0.0156	0.0000	0.0167	0.0000	0.0167	0.0167	0.0312	0.0000	0.0000	0.0167	0.0667	0.0000	0.0000
	18	176	0.0185	0.0806	0.0156	0.0000	0.0000	0.0577	0.0500	0.0167	0.0156	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000
	19	178	0.0000	0.0323	0.0156	0.1094	0.0167	0.0385	0.0167	0.0333	0.0312	0.0167	0.0806	0.0500	0.0833	0.0500	0.0167

	20	180	0.0185	0.0000	0.0000	0.0000	0.0333	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0167
	21	182	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1000	0.0000	0.0000	0.0161	0.0000	0.0167	0.0167	0.0000
	22	184	0.0185	0.0000	0.0000	0.0156	0.0167	0.0000	0.0000	0.0000	0.0000	0.0167	0.0323	0.0333	0.0333	0.0333	0.0167
	23	186	0.0000	0.0000	0.0000	0.0000	0.0000	0.0385	0.0167	0.0000	0.0000	0.0000	0.0161	0.0000	0.0167	0.0167	0.0000
	24	188	0.0000	0.0000	0.0312	0.0000	0.0000	0.0000	0.0167	0.0000	0.0156	0.0167	0.0323	0.0167	0.0000	0.0333	0.0000
	25	190	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0312	0.0000	0.0000	0.0500	0.0000	0.0000	0.0167
	26	192	0.0000	0.0000	0.0312	0.0000	0.0333	0.0000	0.0000	0.0000	0.0156	0.0167	0.0161	0.0167	0.0000	0.0000	0.0167
	27	194	0.0370	0.0000	0.0156	0.0000	0.0000	0.0000	0.0167	0.0000	0.0312	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	28	196	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0167
	29	198	0.0370	0.0000	0.0000	0.0156	0.0167	0.0192	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	30	200	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0167	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	31	202	0.0185	0.0000	0.0000	0.0000	0.0000	0.0192	0.0167	0.0167	0.0000	0.0167	0.0000	0.0167	0.0000	0.0000	0.0000
	32	204	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	33	206	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	34	208	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ploycor 23	1	191	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000
	2	193	0.0185	0.0323	0.0000	0.0000	0.0333	0.0000	0.0000	0.0167	0.0185	0.0517	0.0000	0.0179	0.0500	0.0517	0.0167
	3	197	0.0185	0.0645	0.1167	0.0161	0.0667	0.0400	0.0161	0.0667	0.0926	0.0690	0.0484	0.0357	0.1000	0.0345	0.0500
	4	199	0.1481	0.2581	0.1500	0.1290	0.2667	0.1200	0.1935	0.2000	0.1296	0.1207	0.0968	0.1250	0.0833	0.2069	0.2167
	5	201	0.0370	0.0161	0.0000	0.0323	0.0000	0.0200	0.0161	0.0167	0.0370	0.0345	0.0323	0.0357	0.1667	0.0345	0.0000
	6	203	0.0370	0.0000	0.0500	0.0161	0.1000	0.0800	0.0484	0.0667	0.0370	0.0000	0.0323	0.0179	0.0167	0.0517	0.0833
	7	205	0.0926	0.0806	0.0167	0.0484	0.0500	0.0400	0.0323	0.0500	0.0926	0.0345	0.0645	0.0893	0.0333	0.0862	0.0000
	8	207	0.0185	0.0323	0.0500	0.0323	0.0167	0.0000	0.0000	0.0167	0.1111	0.0345	0.0000	0.0357	0.0500	0.0172	0.1167
	9	209	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0645	0.0000	0.0370	0.0000	0.0000	0.0179	0.0167	0.0000	0.0000

10	211	0.1111	0.0484	0.1833	0.0806	0.0667	0.0400	0.0323	0.0167	0.0926	0.0345	0.0161	0.0714	0.0333	0.0345	0.0000
11	213	0.0556	0.0000	0.0500	0.0000	0.0167	0.0200	0.0161	0.0000	0.0000	0.0000	0.0645	0.0357	0.0000	0.0345	0.0000
12	215	0.0000	0.0161	0.0000	0.0000	0.0167	0.0200	0.0000	0.0333	0.0185	0.0000	0.0000	0.0000	0.0833	0.0000	0.0333
13	217	0.0185	0.0000	0.0000	0.0000	0.0167	0.0200	0.0645	0.0000	0.0000	0.0345	0.0161	0.0893	0.0167	0.0517	0.0000
14	219	0.0000	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0357	0.0000	0.0000	0.0000
15	221	0.0000	0.0161	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0357	0.0333	0.0000	0.0167
16	223	0.0000	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000
17	225	0.0185	0.0161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
18	227	0.0000	0.0161	0.0000	0.0323	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
19	241	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000	0.0000	0.0000
20	263	0.0185	0.0000	0.0500	0.0000	0.0000	0.0000	0.0323	0.0500	0.0185	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000
21	265	0.0370	0.0323	0.0167	0.0161	0.0000	0.0200	0.0161	0.0167	0.0000	0.0517	0.0000	0.0000	0.0000	0.0690	0.0167
22	267	0.0000	0.0323	0.0000	0.0000	0.0167	0.0000	0.0161	0.0333	0.0000	0.0172	0.0000	0.0000	0.0667	0.0000	0.0167
23	269	0.0185	0.0161	0.0000	0.0161	0.0167	0.0800	0.0484	0.0333	0.0000	0.0172	0.0161	0.0357	0.0667	0.0172	0.0000
24	271	0.0370	0.0323	0.0000	0.0000	0.0000	0.0000	0.0161	0.0833	0.0000	0.0345	0.0484	0.0000	0.0000	0.0172	0.0333
25	273	0.0000	0.0161	0.0167	0.0484	0.0000	0.0200	0.0161	0.0333	0.0741	0.0000	0.0161	0.0000	0.0000	0.0517	0.0500
26	275	0.0370	0.0161	0.0000	0.0000	0.0167	0.0400	0.0645	0.0000	0.0370	0.0172	0.0323	0.0000	0.0000	0.0172	0.0333
27	277	0.1481	0.1129	0.0833	0.1452	0.1333	0.0800	0.1129	0.0667	0.0741	0.1724	0.2419	0.0893	0.0333	0.0345	0.1167
28	279	0.0741	0.0806	0.1000	0.2419	0.1000	0.2000	0.0484	0.1500	0.0556	0.1724	0.1613	0.1429	0.0500	0.1552	0.1333
29	281	0.0370	0.0484	0.0167	0.0323	0.0167	0.0800	0.0323	0.0333	0.0185	0.0345	0.0323	0.0714	0.0667	0.0172	0.0167
30	283	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0484	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
31	285	0.0000	0.0000	0.0000	0.0000	0.0167	0.0800	0.0323	0.0000	0.0000	0.0345	0.0161	0.0000	0.0167	0.0000	0.0333
32	287	0.0000	0.0000	0.0167	0.0806	0.0000	0.0000	0.0000	0.0000	0.0000	0.0345	0.0323	0.0000	0.0000	0.0000	0.0000
33	289	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167
34	293	0.0000	0.0000	0.0167	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

	35	295	0.0000	0.0161	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	36	297	0.0185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Polycor 26	1	161	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000
	2	171	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	3	173	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	4	175	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5	177	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000
	6	179	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000	0.1333	0.0000	0.0000	0.0172	0.0000	0.0370	0.0000	0.0000	0.0208
	7	183	0.0000	0.0385	0.0156	0.0000	0.0000	0.0192	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0200	0.0000	0.0000
	8	189	0.0000	0.0000	0.0156	0.0000	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000
	9	191	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0345	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	10	193	0.0000	0.0000	0.0000	0.0000	0.0000	0.0192	0.0167	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000
	11	195	0.0000	0.0000	0.0000	0.0000	0.0556	0.0385	0.0167	0.0000	0.0000	0.0172	0.0000	0.0370	0.0000	0.0000	0.0000
	12	197	0.0345	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	13	205	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.1000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	14	207	0.0172	0.0000	0.0000	0.0333	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000	0.0000	0.0000
	15	209	0.0345	0.0385	0.0469	0.0333	0.0370	0.0769	0.0167	0.0000	0.0000	0.0000	0.0000	0.0741	0.0400	0.0714	0.0000
	16	211	0.0000	0.0000	0.0312	0.0000	0.0000	0.0000	0.0333	0.0167	0.0172	0.0000	0.0000	0.0370	0.0000	0.0000	0.0000
	17	213	0.0345	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0172	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000
	18	215	0.0172	0.0385	0.0000	0.0000	0.0000	0.0577	0.0000	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000	0.0000	0.0625
	19	217	0.0345	0.0769	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	20	219	0.0345	0.0000	0.0312	0.0833	0.0000	0.0192	0.0000	0.0333	0.0172	0.0517	0.0000	0.0000	0.0600	0.0536	0.0000
	21	221	0.0172	0.0000	0.0000	0.0000	0.0000	0.0577	0.0167	0.0000	0.1034	0.0345	0.0000	0.0000	0.0000	0.0357	0.0000
	22	223	0.0000	0.0385	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

23	225	0.0517	0.0000	0.0000	0.0333	0.0000	0.0577	0.0000	0.0167	0.0000	0.0172	0.0370	0.0185	0.0000	0.0357	0.0208
24	227	0.0000	0.0000	0.0312	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0185	0.0400	0.0000	0.0000
25	229	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000
26	231	0.0000	0.0000	0.0312	0.0000	0.0556	0.0385	0.0167	0.0167	0.0000	0.0517	0.0000	0.0741	0.0000	0.0000	0.0000
27	233	0.0000	0.0192	0.0000	0.0167	0.0000	0.0000	0.0167	0.0167	0.0172	0.0345	0.0000	0.0000	0.0800	0.0179	0.0000
28	235	0.0000	0.0385	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0345	0.0185	0.0000	0.0000	0.0000	0.0000
29	237	0.0000	0.0192	0.0000	0.0000	0.0185	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0556	0.0000	0.0000	0.0000
30	239	0.0172	0.0000	0.0625	0.0000	0.0000	0.0000	0.0333	0.0000	0.0000	0.0517	0.0000	0.0370	0.0000	0.0000	0.0417
31	241	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0167	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000
32	243	0.0000	0.0385	0.0000	0.0167	0.0185	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000	0.0714	0.0417
33	245	0.0345	0.0192	0.0312	0.0333	0.0370	0.0192	0.0833	0.0667	0.0345	0.0172	0.0000	0.0370	0.0000	0.0179	0.0833
34	247	0.0345	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0690	0.0000	0.0000	0.0000	0.0600	0.0000	0.0208
35	249	0.0172	0.0000	0.0156	0.0167	0.0185	0.0000	0.0333	0.0000	0.0517	0.0172	0.0926	0.0000	0.0200	0.0000	0.0208
36	251	0.1034	0.0769	0.0625	0.1667	0.0741	0.0577	0.0167	0.0833	0.0690	0.0517	0.1296	0.0556	0.0400	0.1071	0.1667
37	253	0.0172	0.0000	0.0156	0.0000	0.0370	0.0000	0.0167	0.0000	0.0345	0.0172	0.0185	0.0370	0.0000	0.0000	0.0208
38	255	0.0172	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0417
39	257	0.0517	0.0385	0.0469	0.0000	0.0556	0.0000	0.0167	0.0167	0.0345	0.0000	0.0370	0.0926	0.0200	0.0000	0.0000
40	259	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0500	0.0000	0.0345	0.0000	0.0000	0.0000	0.0000	0.0000
41	261	0.0172	0.0192	0.0938	0.0667	0.0185	0.0000	0.0333	0.0000	0.0000	0.0172	0.0000	0.0000	0.1400	0.0536	0.0417
42	263	0.0517	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0172	0.0172	0.0000	0.0185	0.0000	0.0000	0.0000
43	265	0.0517	0.0000	0.0156	0.0000	0.0000	0.0385	0.0000	0.0500	0.0172	0.0172	0.0370	0.0185	0.0400	0.0536	0.1250
44	267	0.0172	0.0577	0.0156	0.0000	0.0556	0.0000	0.1000	0.0167	0.0000	0.0172	0.0370	0.0185	0.0200	0.0000	0.0000
45	269	0.0690	0.0577	0.0000	0.0667	0.0000	0.0192	0.0833	0.0333	0.0172	0.0345	0.0556	0.0370	0.0800	0.0000	0.0208
46	271	0.0000	0.0000	0.0781	0.0000	0.0741	0.0192	0.0167	0.0167	0.0000	0.0690	0.0370	0.0370	0.0000	0.0357	0.0000
47	273	0.0172	0.0577	0.0469	0.0667	0.0556	0.1154	0.0167	0.0833	0.0690	0.0517	0.0370	0.0741	0.0000	0.0179	0.0208

48	275	0.0345	0.0000	0.1094	0.1500	0.0000	0.0000	0.0000	0.0167	0.0000	0.0172	0.0000	0.0185	0.0200	0.0536	0.0000
49	277	0.0517	0.0385	0.0000	0.0000	0.0000	0.0000	0.0833	0.0000	0.0690	0.0517	0.0185	0.0000	0.0000	0.0536	0.0625
50	279	0.0172	0.0385	0.0312	0.0167	0.0370	0.0962	0.0167	0.0333	0.0345	0.0517	0.0185	0.0185	0.0600	0.0714	0.1042
51	281	0.0690	0.0769	0.0312	0.0667	0.0741	0.0769	0.0167	0.1333	0.0345	0.0172	0.0556	0.0185	0.0400	0.0714	0.0000
52	283	0.0000	0.0385	0.0156	0.0000	0.0370	0.0000	0.0167	0.0167	0.0517	0.0000	0.1852	0.0370	0.0200	0.0000	0.0208
53	285	0.0000	0.0192	0.0156	0.0333	0.0000	0.0192	0.0000	0.0000	0.0000	0.0172	0.0185	0.0000	0.0800	0.0179	0.0000
54	287	0.0000	0.0000	0.0469	0.0167	0.0370	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0800	0.0179	0.0000
55	289	0.0172	0.0385	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0185	0.0000	0.0357	0.0000
56	291	0.0000	0.0000	0.0000	0.0000	0.0370	0.1154	0.0000	0.0333	0.0172	0.0345	0.0185	0.0000	0.0000	0.0179	0.0000
57	293	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0333	0.0000	0.0517	0.0000	0.0556	0.0000	0.0000	0.0000	0.0208
58	295	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167	0.0333	0.0690	0.0000	0.0000	0.0000	0.0000	0.0000	0.0417
59	297	0.0000	0.0000	0.0000	0.0500	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
60	299	0.0000	0.0000	0.0000	0.0167	0.0370	0.0000	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000	0.0200	0.0000	0.0000
61	301	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0345	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000
62	303	0.0000	0.0385	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
63	305	0.0000	0.0192	0.0000	0.0000	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
64	309	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
65	315	0.0000	0.0000	0.0000	0.0000	0.0185	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0200	0.0357	0.0000
66	317	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000
67	319	0.0172	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0179	0.0000
68	321	0.0000	0.0000	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Complete list of publications

Peer-reviewed articles (published)

Brückmann, S.V., Krauss, J. & Steffan-Dewenter, I. (2010) Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes

Brückmann, S.V., Krauss, J., van Achterberg, C. & Steffan-Dewenter, I. (2010) The impact of habitat fragmentation on trophic interactions of the monophagous butterfly *Polyommatus coridon*

Habel, J.C., **Brückmann, S.V.**, Krauss, J., Schwarzer, J., Weig, A., Husemann, M. & Steffan-Dewenter, I. (2015) Fragmentation genetics of the grassland *Polyommatus coridon*: Stable genetic diversity or extinction debt?

Presentations

Brückmann, S., Krauss, J., Steffan-Dewenter, I. (2008): Impact of local and surrounding landscape predictors on butterfly diversity in semi-natural grasslands; Future of butterflies in Europe II, International Symposium, Wageningen

Brückmann, S., Krauss, J., Steffan-Dewenter, I. (2008): Habitat connectivity affects butterfly diversity and community composition in calcareous grasslands; Eureco-GfÖ 2008, Tagung, Leipzig

Poster

Brückmann, S., Krauss, J., Steffan-Dewenter, I. (2008): Impact of local and surrounding landscape characteristics on butterflies in calcareous grasslands; BayCEER Workshop 2008, Bayreuth

Manuscripts in this thesis and individual contributions

The studies described in this thesis refer to the following three manuscripts. Manuscript 1 is published in the *Journal of Applied Ecology* (Chapter 5). Manuscript 2 is published in *Journal of Insect Conservation* (Chapter 6). Manuscript 3 is published in *Conservation Genetics* (Chapter 7).

Manuscript 1 (Chapter 5)

Title	**Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes**
Authors	S.V. Brückmann, J. Krauss, I. Steffan-Dewenter
Journal	*Journal of Applied Ecology*
Status	published

Authors contributions

S.V. Brückmann	Design, methods, data collection, analysis, discussion, manuscript writing and editing (corresponding author)
J. Krauss	Design, analysis, discussion, manuscript editing
I. Steffan Dewenter	Design, discussion, manuscript editing

Manuscript 2 (Chapter 6)

Title	**The impact of habitat fragmentation on trophic interactions of the monophagous butterfly *Polyommatus coridon***
Authors	S.V. Brückmann, J. Krauss, C. van Achterberg, I. Steffan-Dewenter
Journal	*Journal of Insect Conservation*
Status	published

Authors contributions

S.V. Brückmann	Design, methods, data collection, analysis, discussion, manuscript writing and editing (corresponding author)
J. Krauss	Design, discussion, manuscript editing
C. van Achterberg	Identification of parasitoid species, manuscript editing
I. Steffan Dewenter	Design, discussion, manuscript editing

Manuscript 3 (Chapter 7)

Title	**Fragmentation genetics of the grassland butterfly *Polyommatus coridon:* Stable genetic diversity or extinction debt?**
Authors	J.C. Habel, S.V. Brückmann, J. Krauss, J. Schwarzer, A. Weig, M. Husemann, I. Steffan-Dewenter
Journal	*Conservation Genetics*
Status	published

Authors contributions

J.C. Habel	Population genetic analysis, discussion, manuscript writing
S.V. Brückmann	Design, methods, data collection, population genetic analysis, discussion, manuscript writing and editing (corresponding author)
J. Krauss	Design, discussion, manuscript editing
J. Schwarzer	Population genetic analysis, manuscript writing and editing
A. Weig	Preparation of genetic material (PCR)
M. Husemann	Calculation census population size, manuscript editing
I. Steffan Dewenter	Design, discussion, manuscript editing

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Many thanks!

Sabrina Brückmann

Bayreuth, October 2014

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